Q fever during pregnancy

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Lessons from the Dutch epidemic

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	Tsjitske Toering

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General introduction and outline of the thesis

GENERAL INTRODUCTION

…''steep increase human Q fever in The Netherlands''
''it is possible that Q fever is endemic in Brabant; other regions should also be alert''
''an association has been found between the human Q fever cases and intensive goat farming in this region''
''this situation raises the question whether the government can and should take preventive measures to prevent human disease''
''questions raise about the need to screen asymptomatic pregnant women''
''knowledge is lacking, but the Dutch situation is an opportunity to provide answers to a wide range of questions''…

…some statements selected from the advice written by the Health Council of The Netherlands to the Ministry of Health, Welfare and Sport at the end of 2008.[1] What kind of disease is threatening us? And what are the risks and implications for pregnant women in specific?

Query fever

Query (Q) fever is a zoonosis, caused by the bacterium *Coxiella burnetii*. It was first investigated by Derrick and colleagues after an outbreak in abattoir workers in Brisbane, Queensland, Australia in 1935. Derrick proposed the term ''Q fever'' (for query fever) to describe this febrile illness caused by an unknown microorganism.[2] He attempted to isolate the etiological agent of the disease, but did not succeed. Derrick sent some infectious material to his college Burnet, who continued the quest. Independently of the group of Burnet, Cox and colleagues were investigating the ecology of Rocky Mountain spotted fever in Montana, USA. The connection between the groups in Montana and Brisbane arose when a laboratory-acquired Q fever infection occurred in the Rocky Mountain Laboratory in 1938. Cox and Burnet were the first who identified the etiological agent of Q fever as a new rickettsial species, which from then on was named *Rickettsia burnetii*. Later this was changed to *Coxiella burnetii*, a name that honours both researchers.[3]

Coxiella burnetii

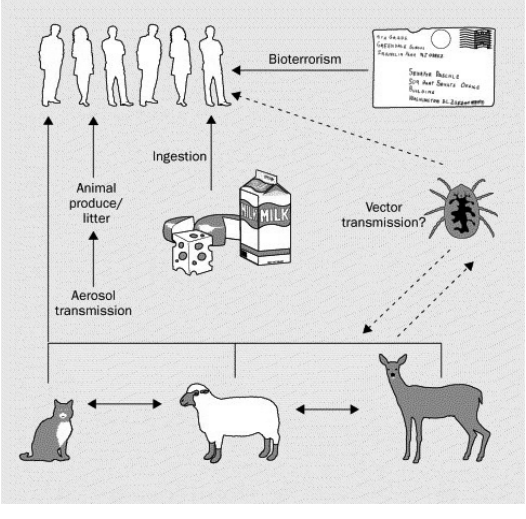
C. burnetii is a small gram-negative intracellular living bacterium that is prevalent throughout the world.[3] Domestic ruminants are considered to be the main reservoir for Q fever in humans, although other animal species, including pet animals, birds and reptiles may also be responsible for human cases.[3] The

role of vectors, especially tics is unclear (Fig. 1).[4] Human-to-human transmission is very rare. There are a few cases described in the literature on maternal-foetal transmission across the placenta[5], transmission by amniotic fluid and placental tissue[6], trough breastfeeding[7], blood transfusion[8], or sperm.[9] The main route for transmission is the respiratory route, in which alveolar macrophages in the lungs are likely the first cells to be infected. A small part of people with Q fever is infected by the digestive route with Kupffer cells in the liver as the target cells.[3] After passive entry in the host cells using specific receptors like integrins, *C. burnetii* enters phagocytic vesicles which fuse rapidly to phagolysosomes. The phagolysosomes fuse progressively to form a large acidic vacuole. *C. burnetii*'s metabolism and multiplication is enhanced by the acidic pH in this vacuole and will be stopped with increasing the phagolysosomal pH.[10]

C. burnetii is characterised by antigenetic phase variation, which is mainly caused by mutational variation in the lipopolysaccharide (LPS).[11] Phase I is highly infectious and is the natural phase found in infected animals and humans. Phase II is less infectious and is obtained only in laboratories after serial passages in cell or embryonated egg cultures.[3] In humans this antigenetic phase variations is especially important in serodiagnosis, which will be discussed later in this introduction.

Encysted ''spores'' of *C. burnetii* have the ability to survive for prolonged periods in dry environmental dust and are highly resistant to disinfectants. Together with the low infective dose, airborne transmission, easily accessible sources and the ability to cause serious illness in large groups of people, *C. burnetii* has been considered a potential weapon for bioterrorism.[4]

Figure 1. Potential routes of transmission of Q fever



Reprinted from Cutler SJ, Paiba GA, Howells J, Morgan KL. Q fever – a forgotten disease? Lancet. 2002;2:717-718. Copyright (2002), with permission from Elsevier.

Clinical signs and symptoms

Following exposure to *C. burnetii*, after an incubation time ranging from 1 to 3 weeks[12], non-immune persons may develop an acute primary infection that is asymptomatic in 60% of the cases. A majority of symptomatic patients experience a mild self-limiting flu-like illness or isolated fever. Others present with atypical pneumonia or hepatitis.[13] Meningitis, meningioencephalitis, myocarditis and pericarditis have also sporadically been described.[14] Besides acute infections *C. burnetii* also has the ability to induce chronic infections, characterised by endocarditis in 78% of the cases.[13] The severity of disease largely varies between hosts. Younger age and female sex seem to be protective factors for symptomatic disease[15,16], whereas immunocompromised patients, patients with cardiac valve or vascular diseases and pregnant women have been reported to have an increased risk to develop chronic Q fever.[3]

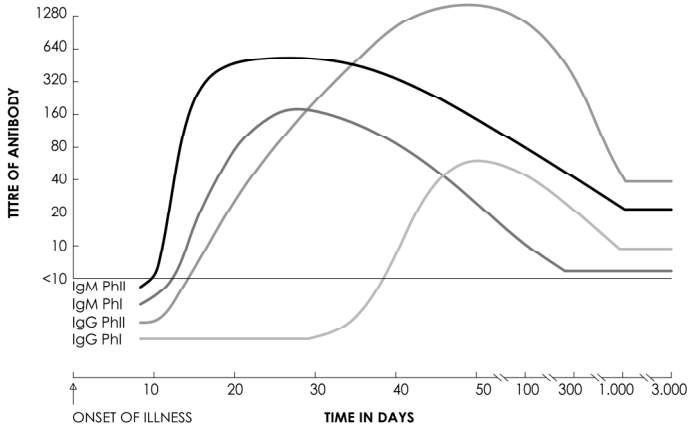
Diagnosis of Q fever

There are several methods to diagnose Q fever including culture, DNA amplification and several serological assays. There is a great difference in simplicity and safety between these methods.[17] Since *C. burnetii* is very infectious, isolation of the bacterium should only be performed in biosafety level 3 laboratories. Under these cicumstances, the process of inoculation and isolation is laborious and therefore not part of the standard diagnostic work up. Polymerase chain reaction (PCR) has successfully been used to detect *C. burnetii* DNA in clinical samples.[18] PCR results are only positive in a short period after the primary infection and during certain phases of chronic Q fever. PCR is therefore especially useful in early diagnosis of both acute and chronic Q fever, but can not be used in the setting of screening.[19]

Because of its simplicity and safety, in most instances the diagnosis of Q fever relies upon serology. There are several serological assays, including micro-agglutination, complement fixation, enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA).[17] Currently, IFA is the reference method.[20] Since one of the characteristics of *C. burnetii* is antigenetic phase variation, antibodies against the two phases can be distinguished.[20] In acute Q fever IgM antibodies against phase II antigens are the first to appear, followed by IgM phase I and IgG phase II in one to two weeks. Finally, weeks to months after the primary infection IgG phase I will appear. All antibodies may persist for many months to even years (Fig. 2).[21,22] Persisting high levels of IgG phase I, mostly in combination with high IgG phase II antibodies, are suggestive for chronic Q fever infection.[20,23] Due to this

timeframe distinguishing previous, acute and chronic infections is possible.

Figure 2. Idealised antibody responses measured by indirect immunofluorescence assay.



PCR, polymerase chain reaction. Adapted from: Marmion BP. Q fever: Your Questions answered. St Leonards, N.S.W, MediMedia Communications, 1999.

Treatment

Since *C. burnetii* is an intracellular living bacterium treatment is challenging. In the general population the first choice treatment for symptomatic acute Q fever consists of doxycycline 100mg twice a day for at least 14 days.[3,24] In a randomised controlled trial[25] and in retrospective studies, doxycycline outperformed other antibiotics including erythromycin.[26,27] Research on newer macrolides and fluoroquinolones looks promising but has not ended yet.[26,28,29] In case of chronic Q fever with endocarditis antibiotic treatment recommendations vary from 18 months to life-long. Doxycycline should be combined with the lysosomotropic agent hydroxychloroquine to increase the efficacy of doxycycline bij increasing the phagolysosomal pH.[3,30] Whether asymptomatic serological profiles suggesting chronic Q fever, without cardiovascular or other physical complications, should be treated, is not clear.

Human vaccine prophylaxis

Despite that there are several animal vaccines available, there is only one Q fever vaccine (Q-Vax, Commonwealth Serum Laboratories Limited) available for humans. This vaccine is registered in Australia and is given to the Australian population with the highest occupational exposure to *C. burnetii* (mainly abattoir workers).[3] Although highly immunogenic, this vaccine may induce

adverse effects, especially when administered in previously infected persons.[31] Since efficacy and safety have predominantly been investigated in the specific group of abattoir workers[32,33], this crucial information is lacking for the general population and for specific groups at risk, including pregnant women.

Q fever during pregnancy

Both symptomatic and asymptomatic *C. burnetii* infection during pregnancy have been associated with adverse pregnancy outcomes.[5,34,35] A milestone hospital-based study from France showed that 81% of the pregnant women with untreated Q fever had a miscarriage, premature delivery, intrauterine growth restriction or foetal death. Furthermore, 50% of the maternal infections were followed by a chronic infection, in 10% with *C. burnetii* endocarditis. These complications seemed to be related to placental infection with *C. burnetii*. Both obstetric and maternal complications were found to occur more often in pregnant women infected during their first trimester of pregnancy than in those infected later.[34] In another study from Canada *C. burnetii* seropositive parturient women had a twice as high risk for adverse pregnancy outcome including premature delivery and prior or current neonatal death.[35] These figures are alarming and emphasise that *C. burnetii* infection is a potential threat to pregnant women and their foetus. Long-term antibiotic treatment during pregnancy has been shown to deminish *C. burnetii* related complications.[34] Cotrimoxazole for at least five weeks has been put forward as the first choice treatment during pregnancy, since doxycycline and hydroxychloroquine are contraindicated from the second trimester of pregnancy.[34]

Since most of the pregnant women with a *C. burnetii* infection remain asymptomatic (up to 90% compared to 60% in the general population[13,36]) and infections during as well as prior to pregnancy may lead to complications, preventive policies based on clinical symptoms are not useful. Instead, routine serological screening during pregnancy in endemic areas for Q fever could be of great value to prevent complications in this potential high-risk group, but evidence from randomised trials is lacking.

The Dutch Q fever epidemic

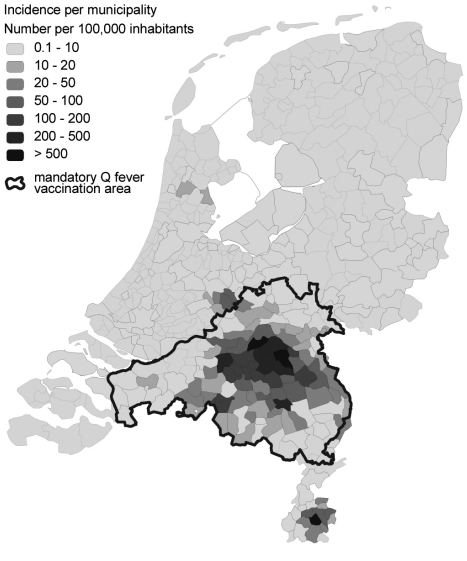
In The Netherlands Q fever became a notifiable disease in 1978. However, prior to 2007 the number of notifications of symptomatic (fever, pneumonia or hepatitis), laboratory confirmed cases was low; between 10 and 20 cases each year.[37] In 2007 these numbers briskly increased many-fold to 168.[38] A human outbreak around an infected goat farm in the Southeast was held

responsible for this increase.[39,40] In the following years there was a steep increase in notified cases to over 2300 in 2009 and the high-risk area in the South-eastern part of The Netherlands expanded to adjacent regions (Fig. 3).[41] Because of an increased awareness of Q fever among general practitioners, diagnostic bias may in part have led to the high number of notified cases in 2008 and 2009. However the major cause of the increase seems to be the increasing numbers of infected dairy goat and sheep farms causing both symptomatic and asymptomatic human cases.[42] Other European countries, such as Belgium, Cyprus and Germany also reported an increasing number of Q fever cases since 2007, although to a much smaller extent.[43]

The enormous magnitude of the Dutch outbreak led to several meetings of the Dutch Outbreak Management Team (OMT) and the Health Council of The Netherlands. Preventive measures in general and for risk groups, like pregnant women, in specific were discussed to curb the epidemic. At the end of 2008 the Health Council advised the Ministry of Health, Welfare and Sport to facilitate studies to inform decision makers about the possible value of screening pregnant women for *C. burnetii* infection. They considered the results of previous studies on *C. burnetii* associated risks to be alarming, but information on the prevalence in, and the potential benefits and harms associated with screening of this potential high-risk group, were lacking.[1]

This thesis will focus on the topic of screening for *C. burnetii* infection during pregnancy. The main objective was to assess the effectiveness of large-scale routine serological screening for *C. burnetii* infection during pregnancy in Q fever high-risk areas.

Figure 3. Human Q fever incidence/100,000 inhabitants per municipality in The Netherlands, 1 January-12 August 2009.



The dark line shows the dairy goat and dairy sheep mandatory vaccination area in 2009. (Compiled by the National Institute for Public Health and the Environment (RIVM))

OUTLINE OF THE THESIS

The studies in this thesis will discuss the lessons we can learn from the Dutch Q fever epidemic between 2007 and 2010, focusing on infection during pregnancy. It starts in **Chapter 2** at the level of the consulting room, with one pregnant woman with chronic Q fever. We describe the problems of this individual patient and discuss the challenges for here caregivers, substantiated with theoretical background. The thesis will continue with the large regional Q fever outbreak and the emerging questions concerning this infection during pregnancy. What are the risks? Is there evidence to promote routine screening of asymptomatic pregnant women? And are tests and treatment available? In **Chapter 3** we try to give answers based on a literature search. Subsequently, the study protocol and results of our clustered randomised controlled trial on the effectiveness of routine screening for Q fever during pregnancy are presented (**Chapter 4** and **Chapter 5**). In **Chapter 6** the focus is on the role of positive *Coxiella burnetii* serology in the prediction of adverse pregnancy outcomes. After these epidemiological based studies, **Chapter 7** and **Chapter 8** concern more basic research on placental histopathology after *C. burnetii* infection and specificity of the indirect immunofluorescence assay, respectively. Since the success of a screening program will not only be based on medical effectiveness, **Chapter 9** focuses on risk perception and the psychological aspect concerning Q fever during pregnancy. We aim to identify determinants which are crucial in the decision of pregnant women to participate in a (fictional) Q fever screening program. Finally, in **Chapter 10** a meta-analysis on the effectiveness of human Q fever vaccination, a more preventive strategy to curb the epidemic, is presented. The thesis will end with a general discussion (**Chapter 11**).

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Chapter 2

Chronische Q-koorts tijdens de zwangerschap (casuïstiek)

Nederlands Tijdschrift voor Geneeskunde 2011;155:A2781.

Extended abstract in English on page 28.

Janna M. Munster
Carl J.C.M. Hamilton
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ABSTRACT

Een 42-jarige vrouw werd ter controle van een doorgemaakte pneumonie gezien door de longarts. Die constateerde dat de pneumonie een uiting was geweest van een acute Q-koortsinfectie. Enkele weken later bleek patiënte onverwacht zwanger te zijn. Bij de reguliere serologische follow-up 6 maanden na de primaire infectie werd de diagnose 'chronische Q-koorts' gesteld. Voor behandeling met doxycycline en hydroxychloroquine was er een contra-indicatie vanwege de zwangerschap en patiënte bleek allergisch te zijn voor co-trimoxazol. Op empirische gronden werd daarom gekozen voor behandeling met erytromycine. Patiënte ervoer veel klachten tijdens de zwangerschap. Op maternale indicatie werd de bevalling bij een amenorroeduur van 38 weken en 2 dagen ingeleid. Patiënte beviel uiteindelijk middels sectio caesarea van een gezonde zoon van 3850 g. In verband met een verhoogd risico op chronische Q-koorts tijdens de zwangerschap, adviseren wij ook na een acute infectie vlak vóór de zwangerschap de serologische controles te intensiveren.

INTRODUCTIE

Sinds 2007 kampt Nederland met een Q-koortsuitbraak van ongekende omvang.[1] Acute Q-koorts wordt gekenmerkt door koorts, hepatitis of pneumonie, maar kan ook asymptomatisch verlopen. De ziekte wordt veroorzaakt door de bacterie *Coxiella burnetii.* Q-koorts kan leiden tot een chronisch ziektebeeld, waarbij endocarditis of infecties van vasculaire structuren ontstaan. De kans op het ontwikkelen van chronische Q-koorts is groter bij immuungecompromitteerden, patiënten met pre-existent klep- of vaatlijden en zwangeren.[2] Eerder verscheen in het *Tijdschrift* een artikel over een vaatpatiënt met chronische Q-koorts.[3] Naast het verhoogde risico op chronische Q-koorts zijn er bij zwangeren met Q-koorts mogelijk ook risico's voor de foetus, voornamelijk bij infecties vroeg in de zwangerschap.[4] Als deze zwangeren niet worden behandeld, bestaat er een verhoogde kans op abortus, vroeggeboorte, groeirestrictie en intra-uteriene vruchtdood.[5] In dit artikel beschrijven wij de diagnostiek en therapie bij een zwangere met chronische Q-koorts.

ZIEKTEGESCHIEDENIS

Patiënt A, een 42-jarige vrouw, bezocht ter controle de longarts na het doormaken van een pneumonie. De arts vond geen bijzonderheden, behalve positieve serologische uitslagen voor *C. burnetii*. Patiënte vertelde daarop inderdaad in contact te zijn geweest met geiten in een gebied waar Q-koorts voorkwam. De antibiotische therapie die zij inmiddels had voltooid, was niet optimaal voor de behandeling van Q-koorts, maar patiënte ervoer op dat moment geen klachten meer. Er werd een afspraak gemaakt voor de standaard serologische follow-up na 3, 6 en 12 maanden om een chronische infectie uit te sluiten (zie uitlegkader).

Enkele weken laten bleek patiënte onverwacht zwanger te zijn. Bij 15 weken amenorroeduur werd er vanwege haar leeftijd een vruchtwaterpunctie verricht. Er waren geen aanwijzingen voor chromosomale afwijkingen en de PCR op *C. burnetii* in het vruchtwater was negatief. Serologisch onderzoek na 3 maanden toonde geen aanwijzingen voor een chronische infectie. Zes maanden na de primaire infectie (25 weken amenorroe) waren de antilichamen echter fors gestegen (IgG fase I van 1:256 naar 1:4096 en IgG fase II van 1:4096 naar 1:16.384; zie uitlegkader). Daarnaast was de PCR op *C. burnetii* in het serum positief, wat de diagnose 'chronische Q-koorts'

bevestigde.[6] De patiënte kreeg co-trimoxazol, maar reageerde daar allergisch op met roodheid en koorts. Op empirische gronden werd co-trimoxazol vervangen door erytromycine, waarna de serologische waarden niet verder doorstegen. Wel had patiënte in het derde trimester klachten zoals moeheid, dyspneu en gewichtsverlies, die duidelijk ernstiger waren dan in haar voorgaande 2 zwangerschappen. De groei van de foetus was conform de zwangerschapstermijn. Bij een amenorroeduur van 38 weken en 2 dagen werd patiënte op maternale indicatie ingeleid. Bij 3 cm ontsluiting besloot de gynaecoloog op verdenking van foetale nood een secundaire sectio caesarea te verrichten. In verband met mogelijke infectiositeit van de placenta en het vruchtwater gebeurde dit onder strikte hygiënische maatregelen.[5] Het jongentje dat geboren werd woog 3850 g (P_{90}) en maakte een goede start. PCR liet zien dat placenta en vruchtwater positief waren voor *C. burnetii*; navelstrengbloed en perifeer bloed van de pasgeborene waren negatief. Bij het verlosteam werd geen Q-koorts vastgesteld.
Post partum begon patiënte met doxycycline en hydroxychloroquine. Het geven van borstvoeding werd haar afgeraden vanwege mogelijke verticale transmissie van *C. burnetii*.[7] Echoscopie toonde geen endocarditis en vasculaire problematiek aan en een PET-scan toonde geen traceractiviteit in de uterus, wat er op wees dat ook daar geen infectie speelde. Desondanks bleven de antistofuitslagen hoog. Omdat patiënte ernstige dermatologische en gastro-intestinale bijwerkingen had van de combinatietherapie, vervingen wij deze 18 weken post partum door ciprofloxacine. Hierop ontwikkelde patiënte echter ernstige artropathie, waarna besloten werd de behandeling volledig te staken. De serologische waarden bleven onverminderd hoog (1:1064 voor IgG fase I en II), maar er werd geen positieve PCR meer gevonden.
De pasgeborene ontwikkelde zich goed en serologische controles tot 6 maanden post partum toonden slechts dalend maternaal IgG, maar geen tekenen van een actieve Q-koortsinfectie.

BESCHOUWING

Het te voeren beleid bij chronische Q-koorts is nog onvoldoende evidence based. Bij onze casus kwam het beleid tot stand na overleg tussen verschillende disciplines in een centrum waar relatief veel ervaring is met Q-koorts.

Zwangerschap als risicofactor voor chronische Q-koorts

Tijdens een zwangerschap past het immuunsysteem zich aan om tolerantie voor foetale antigenen van vaderlijke origine te bewerkstelligen. Onder invloed van oestrogenen en progestagenen wordt de celgemedieerde immuunrespons onderdrukt.[8] *C. burnetii* is een intracellulair levende bacterie; klaring van de infectie zal daarom voornamelijk afhankelijk zijn van de celgemedieerde immuunrespons,[2] waardoor tijdens de zwangerschap de kans op persisteren van de infectie groter is. Daarnaast is de placenta één van de doelwitorganen van *C. burnetii.* In de veterinaire geneeskunde is bekend dat er bij geïnfecteerde drachtige dieren veelal sprake is van vroeg- en doodgeboorte van jongen wat samengaat met een beeld van placentitis. Placenta en vruchtwater zijn dan zeer infectieus.[9] Ook bij de mens is deze placentitis aangetoond.[5]

Follow-up

Het Jeroen Bosch Ziekenhuis heeft een lokale richtlijn voor de follow-up van alle patiënten die een acute Q-koortsinfectie hebben doorgemaakt. Deze richtlijn adviseerde tot 2010 om 3, 6 en 12 maanden na de primaire infectie serologisch onderzoek te verrichten om chroniciteit van de infectie uit te sluiten.[10] Na evaluatie van de resultaten hiervan, wordt sinds 2010 eenmalig na 9 maanden gecontroleerd. Met de wetenschap dat zwangeren een verhoogd risico hebben op chronische Q-koorts, is het te rechtvaardigen om hen vaker serologisch te vervolgen, ook als zij vlak voor de zwangerschap acute Q-koorts hadden. Zo nodig kan dan behandeling plaatsvinden.

Behandeling

De therapie van eerste keus bij patiënten met chronische Q-koorts is een combinatie van doxycycline en hydroxychloroquine voor minimaal 1 jaar.[2] Er is echter een contra-indicatie voor doxycycline vanaf het tweede trimester van de zwangerschap in verband met vertraging van de osteogenese bij de foetus. Op dit moment is er voor de behandeling van Q-koorts tijdens de zwangerschap de meeste ervaring met het combinatiepreparaat co-trimoxazol. Aanbevolen wordt om minimaal 5 weken te behandelen. Deze aanbeveling is echter gebaseerd op een retrospectieve studie waarbij mogelijke vertekening is van de resultaten door selectiebias.[5] Onze patiënte bleek overigens allergisch te zijn voor co-trimoxazol. Wat het beste middel is als tweede keus voor Q-koorts tijdens de zwangerschap is niet aan te geven. Meerdere middelen die in aanmerking zouden kunnen komen, zijn relatief

gecontra-indiceerd tijdens de zwangerschap, of zijn niet geregistreerd voor zwangeren.

CONCLUSIE

Zwangeren die vlak voor de zwangerschap een acute Q-koorts infectie doormaken, hebben mogelijk meer kans op het ontwikkelen van chronische Q-koorts, net als zwangeren die tijdens de zwangerschap een infectie doormaken. Intensievere serologische follow-up tijdens de zwangerschap lijkt ook bij deze vrouwen aangewezen. De behandeling is niet eenvoudig, temeer omdat er voor de behandeling van eerste keus met doxycycline en hydroxychloroquine een contra-indicatie bestaat vanaf het tweede trimester van de zwangerschap.

Uitleg diagnostiek chronische Q-koorts

De diagnostiek van chronische Q-koorts is gebaseerd op serologisch onderzoek eventueel gevolgd door PCR. *Coxiella burnetii* heeft 2 antigene fasen. Antistoffen tegen fase II-antigenen ontstaan kort na blootstelling aan de bacterie en zijn kenmerkend voor een acute Q-koortsinfectie. Chronische Q-koorts gaat juist gepaard met hogere titers van IgG fase I, meestal samen met hoge titers van IgG fase II. Er is nog geen consensus over de afkapwaarden voor de titers die hierbij gehanteerd dienen te worden, mede omdat deze afhankelijk zijn van de gebruikte test. Om een chronische infectie op te sporen is het dus nodig zowel IgG-antistoffen tegen fase I-antigenen als tegen fase II-antigenen te bepalen.[10] De diagnose wordt bevestigd door een passend klinisch beeld of het aantonen van *C. burnetii*-DNA in bloed of weefsel door middel van PCR-onderzoek.[6]

Leerpunten

- Zwangeren met acute Q-koorts hebben meer kans op een chronische Q-koortsinfectie.
- Het is raadzaam ook zwangeren die kort voor hun zwangerschap een acute Q-koortsinfectie doormaken intensief op een chronische infectie te screenen.
- Vanaf het tweede trimester van de zwangerschap is er een contra-indicatie voor behandeling met doxycycline en hydroxychloroquine, de therapie van eerste keus bij chronische Q-koorts; eventuele alternatieven zijn co-trimoxazol en erytromycine
- De angst voor nadelige effecten van een Q-koortsinfectie op de zwangerschapsduur en op het geboortegewicht van de neonaat en voor intra-uteriene transmissie van *C. burnetii* is niet altijd gegrond.

EXTENDED ABSTRACT

Aim

Since 2007 The Netherlands is suffering from an outbreak of Q fever, caused by *Coxiella burnetii*, with more than 2300 patients in 2009. Besides an acute form, which is characterised by pneumonia, hepatitis or fever, a chronic infection may develop. Immunocompromised patients, patients with underlying cardiac valve- or vessel disease and pregnant women have a higher risk to develop chronic Q fever. The increased risk for pregnant women might be explained by the knowledge that the T-cell response of the immune system is suppressed by high levels of female hormones. Furthermore, the placenta seems to be one of the target organs, since *C. burnetii* causes placentitis in both animals and humans. Besides the risk for chronic Q fever infection, obstetric complications, like miscarriage, intrauterine growth restriction and intrauterine foetal death, have been described. In this article we discuss the difficulties concerning diagnose and treatment of chronic Q fever during pregnancy.

Case description

A 42 years old lady suffered from an acute Q fever infection shortly before her third pregnancy. Regular serological follow-up at 3, 6, and 12 months is arranged. At 15 weeks of gestation (GA) an amniocentesis is performed. There are no chromosomal abnormalities and PCR on *C. burnetii* is negative. Serology at three months shows low antibody titres, however six months after the primary infection (GA 25 weeks) there is a steep increase of IgG phase I (from 1:256 to 1:4096) and IgG phase II (from 1:4096 to 1:16,384) and PCR in serum is positive. Chronic Q fever is diagnosed and treatment with cotrimoxazole is started. However, the patient develops an allergy and cotrimoxazole is empirically switched to erythromycin. Antibody titres do not further increase, but the patient experiences many complaints. Therefore at 38 weeks and two days of gestation labour is induced. Finally, a healthy boy with normal birth weight of 3850 grams is born with a secondary caesarean section. PCR *C. burnetii* on placenta and amniotic fluid are positive. PCR on umbilical cord blood and peripheral blood of the newborn are negative. Erythromycin is switched to doxycycline and hydroxychloroquine. Endocarditis, vascular abnormalities and retentio placentae are excluded with ultrasound and PET scan. However, antibody levels remain high with a negative PCR and the patient experiences many side effects of her treatment. Eighteen weeks post partum combination therapy is therefore switched to ciprofloxacin. Unfortunately, the patient develops ciprofloxacin-related arthropathy.

Therefore, complete cessation of antibiotic therapy is decided. Patients' complaints decrease, however serology remains high.
The newborn develops without any problems and serology shows, besides maternal IgG, no signs of active Q fever infection.

Conclusion

Like an acute Q fever infection during pregnancy an acute infection shortly before pregnancy should be considered as a risk factor for developing chronic Q fever. We advise to intensivate serological follow-up in these cases. Treatment is challenging because first choice treatment with doxycycline and hydroxychloroquine is contraindicated after the first trimester of pregnancy.

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Chapter 3

Screening for *Coxiella burnetii* infection during pregnancy: pros and cons according to the Wilson and Jungner criteria

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ABSTRACT

In Europe the incidence of human Q fever has dramatically increased over the previous years. Untreated infections with *Coxiella burnetii,* the causal agent of Q fever, have been associated with both obstetric and maternal complications. The majority of pregnant women with a *C. burnetii* infection remain asymptomatic, hence screening could be of value to prevent unwanted outcomes in this high-risk group. We applied the updated Wilson and Jungner criteria to review the evidence for routine screening for *C. burnetii* infection during pregnancy. Since much uncertainty remains about the incidence, clinical consequences, diagnostics and treatment of *C. burnetii* infection during pregnancy, routine screening for *C. burnetii* infection during pregnancy should not be recommended. Rigorous studies to assess the effectiveness of *C. burnetii* screening are warranted.

INTRODUCTION

Infections during pregnancy may cause a threat to both maternal and foetal health, even if the infected pregnant woman herself remains asymptomatic.[1] Therefore, routine screening at 12 weeks of gestation is being offered to all Dutch pregnant women for human immunodeficiency virus (HIV), *Treponema pallidum* and hepatitis B virus (HBV). The incidence of human Q fever, a zoonosis caused by *Coxiella burnetii*, showed an enormous increase in The Netherlands and other European countries over the past few years.[2] Since there is evidence for infection-associated obstetric and maternal complications, *C. burnetii* infection poses a potential risk to pregnant women and their (unborn) children.[3] Most of the pregnant women with a *C. burnetii* infection remain asymptomatic.[4] Therefore routine screening has been put forward for early detection and treatment in this group, but scientific evidence about the usefulness of such an intensive program is lacking. In this review we applied the Wilson and Jungner criteria according to the World Health Organization to scrutinise the available evidence for routine screening for *C. burnetii* infection during pregnancy. These criteria were developed over 40 years ago but are still of great value in decision making around screening policies.[5] The criteria centre on the problem caused by the infection or disease, the screening population, the test and the treatment, and the costs. As newer policy tools, especially concerning genetic screening, have been suggested[6], we also integrated the emerging criteria which are applicable to our research question. A review of the literature was done by searching PubMed and the references of included papers. Our search was limited to studies in English or Dutch. The search strategy included the keywords 'Q fever' or '*Coxiella burnetii*' and keywords related to the criteria ('incidence' or 'prevalence' or 'pregnancy' or 'risk factors' or 'diagnosis' or 'treatment' or 'costs'). Our overall aim was to examine the evidence base for routine *C. burnetii* screening among pregnant women in high-risk areas for Q fever all over Europe.

THE PROBLEM

Terminology used in the scientific literature concerning 'Q fever' is diverse and therefore direct comparisons of epidemiological studies should be performed with caution. 'Q fever' is commonly referred to the symptomatic disease, including symptoms such as fever, hepatitis or pneumonia in combination with positive antibody titres or polymerase chain reaction (PCR). The terms 'C.

burnetii infection' and 'presence of antibodies' are more often used in the context of asymptomatic disease, for example, in prevalence studies.

Is *Coxiella burnetii* infection during pregnancy an important health problem?

Prior to 2007 Q fever was uncommon in Europe[2], except from some local outbreaks such as the outbreak in Germany in spring 2005, causing 331 cases.[7] In The Netherlands around 10 to 30 cases have been notified each year since 1977. Between 2007 and 2009 the numbers briskly increased to over 2300 cases in 2009, the highest number ever reported in the literature.[8] Veterinary outbreaks on several dairy goat and sheep farms in the southern parts of The Netherlands are held responsible for this increase. In 2009 and 2010 it was decided to implement extensive measures such as vaccinating and culling of thousands of animals.[8] As a result, the number of human Q fever cases decreased rapidly to around 500 cases by the end of 2010, which is still considerable and may indicate an endemic stage.[9] Also other European countries, such as Belgium, Cyprus and Germany have reported an increasing number of cases since 2007, albeit to a smaller extent.[2]

The prevalence of Q fever among pregnant women is unknown. Recently published data from The Netherlands showed a prevalence of immunoglobulin (Ig)M, suggesting recent infection with *C. burnetii*, in 3.4% of 1646 tested serum samples from pregnant women in Q fever high-risk areas.[10] In a cohort study from Canada, 3.8% of parturient women had evidence of previous exposure to *C. burnetii* (presence of IgG phase I and/or II). These women had, in contrast to the Dutch seropositive women[10], a higher risk for adverse pregnancy outcomes, in terms of premature delivery and prior or current neonatal death, compared with seronegative women.[11] A milestone hospital-based study from France showed that 81% of the pregnant women with untreated Q fever had a miscarriage, premature delivery, intrauterine growth restriction or foetal death. Furthermore, chronic Q fever occurred in 50% of the cases, of whom 10% developed *C. burnetii* endocarditis.[3] These figures are alarming, but need to be cautiously interpreted as the retrospective design covering many years may have led to some overestimation of risks. Certainly, this study together with the prevalence studies emphasise that *C. burnetii* infection is a potential threat to pregnant women.

Is there a latent or early symptomatic stage?

Up to 90% of infected pregnant women remain asymptomatic.[4] Therefore, early detection, before obstetric complications and maternal chronic Q fever

have occurred, enables treatment that may prevent complications due to *C. burnetii* infection.[3]

Is the natural history of *Coxiella burnetii* infection adequately understood?

C. burnetii is a small gram-negative intracellular living bacterium. The main route of transmission is the respiratory route, in which alveolar macrophages in the lungs are the first cells to be infected.[12] Furthermore, the placenta seems to be a target organ since placentitis has been described in both animals and humans.[3,13] After the primary infection, *C. burnetii* has the ability to induce chronic infections. It is hypothesised that, besides the liver, bone, heart valves and mural endocardium[14], the uterus could be a site of latent infection, hence reactivation during pregnancy can occur.[3,11]

The pathogenesis of obstetric complications following infection is not completely understood; immune complexes may cause vasculitis and vascular thrombosis, which in turn may lead to the placental insufficiency and subsequent obstetric complications.[15] Also, direct transplacental transmission by *C. burnetii* may cause foetal death.[16] Obstetric complications occur significantly more often in patients who are infected during the first trimester of pregnancy than in those infected later.[3]

Not only acute infections have been associated with obstetric complications, also previous infections seem to increase the risk.[11] There is no good explanation for this association besides the hypothesis of intrauterine latency of the infection.[11] In all, the natural history of *C. burnetii* infection among pregnant women is not completely understood.

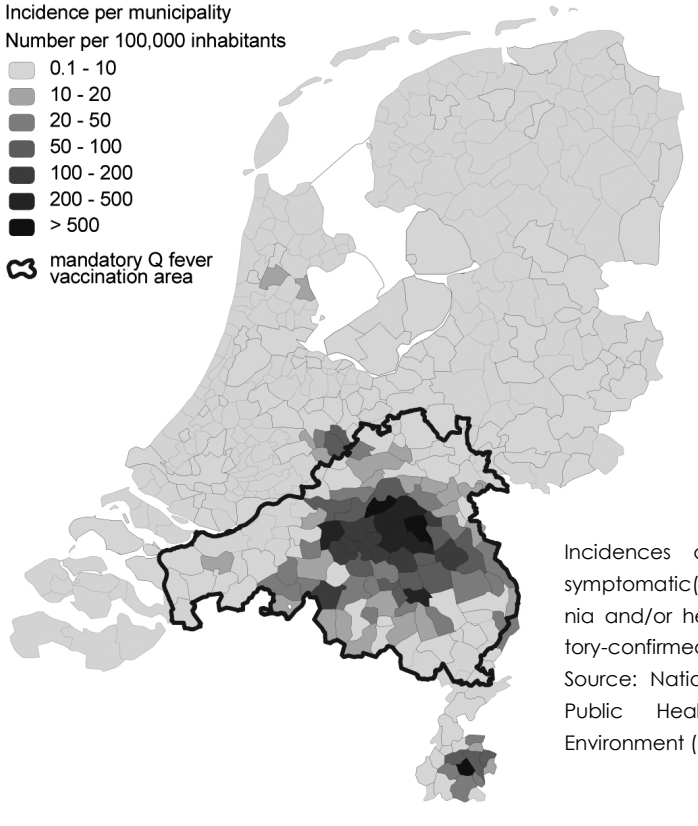
THE SCREENING POPULATION

Since the Q fever incidence largely varies between regions (see for example the situation in The Netherlands, Fig. 1), the population for routine screening should be limited to pregnant women living in high-risk Q fever areas. Women living within a five-kilometre zone around a dairy goat or dairy sheep farm affected by *C. burnetii*-related abortion waves have the highest risk of contracting an infection, however, still 41% of the Dutch cases in 2009 lived outside of these areas.[8] Whether these cases visited the five-kilometre zones is unclear. Therefore, if introduced, routine screening of all pregnant women would be advisable in areas with a high incidence (e.g. >50/100,000

inhabitants). So, with a good surveillance system, the screening population can be accurately defined. Screening of specific groups at risk, e.g. pregnant women with occupational hazard for Q fever or with complicated pregnancies can also be considered, but is beyond the scope of this study discussing routine screening of a total population.

Similar to other screening programs during pregnancy, eligible women have to be counselled about the benefits and possible consequences of the screening (i.e. long-term antibiotic treatment and hospital birth instead of home birth in case of an acute infection, stress induced by awareness of infectious diseases during pregnancy) to be able to make an informed choice about participation.

Figure 1. Human Q fever incidence per 100,000 inhabitants per municipality in The Netherlands, 1 January-12 August 2009.



Incidences are based on symptomatic(fever, pneumonia and/or hepatitis), laboratory-confirmed Q fever cases. Source: National Institute for Public Health and the Environment (RIVM.)

Is there an agreed policy on whom to treat as patients?
All phases of *C. burnetii* infection during pregnancy have been associated with adverse pregnancy outcome. However, evidence for the benefits of antibiotic treatment is only available in patients with acute and chronic Q fever.[3] Whether antibiotic treatment prevents complications in women with asymptomatic seropositivity needs to be investigated.

Is case finding a continuing process and not a "once and for all" project?
If introduced, screening for *C. burnetii* infection should be performed during each pregnancy since the infection can be contracted at any moment and reactivation during pregnancy of a previous infection may occur.[3,11] Therefore case finding is a continuing process.

THE TEST AND THE TREATMENT

Is there a suitable test?
There are several accurate methods to diagnose *C. burnetii* infection, including culture, PCR and serology, of which serology is most suitable for screening.[17] However, the performance of these tests during pregnancy is unknown. In the general population, indirect immunofluorescence assay (IFA) is the reference method.[17,18] Since one of the characteristics of *C. burnetii* is antigenetic phase variation, antibodies against two phases of antigens can be detected. All types of antibodies have their own timeframe of appearance, therefore distinguishing previous, acute and chronic infections is possible.[12,18] As already mentioned, test characteristics during pregnancy are unknown. From other infectious diseases we know that false-positive serological results occur quite often during pregnancy.[19] Furthermore, with respect to sensitivity and specificity, there is an ongoing debate about which cut-off values to use, especially because there are many different commercial and in-house methods. In all, more research needs to be performed with respect to serological screening for *C. burnetii* during pregnancy before routine screening can be implemented.

Is the test acceptable to the population?
Acceptance of the test can be expected since only one blood sample is necessary, which can be obtained by venepuncture combined with the screening for other infectious diseases around 12 weeks of pregnancy. An advantage of testing in the first trimester is the possibility of early counselling

and treatment during the most vulnerable phase of pregnancy.[3] However, with early screening, infections later in pregnancy would be missed. Timing of the screening needs to be further investigated, also taking into account a seasonal variation in *C. burnetii* spreading.[9]

Is there an accepted treatment for patients with recognised disease?

First choice treatment for Q fever among the general population is a 14-day antibiotic treatment with doxycycline or fluoroquinolone.[12] However, both agents are contraindicated during pregnancy. Long-term treatment with cotrimoxazole has been suggested to be the treatment of choice during pregnancy.[3] However, use of cotrimoxazole during pregnancy has not been fully investigated yet. Pharmacological activity of this drug could cause folic acid depletion in the foetus.[20] Furthermore, neonatal hyperbilirubinemia has been described when used prior to delivery. However, these risks turned out to be small in large groups of pregnant women with HIV who received prophylactic cotrimoxazole therapy during pregnancy.[21] In all, more evidence for the best treatment option during pregnancy is needed.

Are there facilities for diagnosis and treatment available?

Since screening for other infectious diseases during pregnancy is already routinely performed, adding *C. burnetii* screening will be relatively straightforward. In The Netherlands, as in other Western countries, several laboratories have facilities to perform *C. burnetii* serology. Quality assessments should be performed on a regular basis. Treatment and follow-up of positively screened women should be performed by obstetricians, infectious disease specialists and medical microbiologist, who should receive additional training on diagnostics and treatment of *C. burnetii* infection during pregnancy.

THE COSTS

Are the costs of case finding economically balanced in relation to possible expenditure on medical care as a whole?

Outcomes of cost-effectiveness models are not available yet and input data are required. Screening with IFA and antibiotic treatment are relatively cheap, though referral for treatment and hospital birth may induce high costs since around 25% of the deliveries in The Netherlands normally take place at home.[22]

The adapted Wilson and Jungner criteria, addressed in this study are summarised in Table 1.

CONCLUSION

According to the adapted Wilson and Jungner criteria (Table 1), the currently available evidence is insufficient to promote routine screening for *C. burnetii* infection during pregnancy in high-risk Q fever areas. Because of potential bias in the studies reported so far, there is too much uncertainty about the consequences of untreated *C. burnetii* infection during pregnancy. There is also no consensus about the screening method and treatment. Furthermore, Q fever incidence rates highly affect the effectiveness of screening. Therefore the candidate populations for screening are not static and should be identified based on epidemiological criteria. Finally, besides screening, there are other methods to prevent *C. burnetii* related complications, for example human vaccination.[23] Overall, more evidence about the effectiveness of a *C. burnetii* screening program, in addition to other Q fever prevention and control measures taken by the European countries, is needed before this infection will become a candidate for routine screening during pregnancy.

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Table 1. Wilson and Jungner criteria and emerging criteria (*Italic*) for disease screening.

Wilson and Jungner criteria and emerging criteria for disease screening	Criteria fulfilled?
<u>The problem</u>	
The condition sought should be an important health problem.	Not certain
There should be a latent or early symptomatic stage.	Yes
The natural history of the condition should be adequately understood.	Not certain
The screening program should respond to a recognised need.	*Not certain*
The objectives of screening should be defined at the outset.	*Yes*
<u>The screening population</u>	
There should be an agreed policy on whom to treat as patients.	Not certain
Case finding should be a continuing process and not a "once and for all" project.	Yes
There should be a defined target population.	*Yes*
The program should ensure informed choice, confidentiality and respect for autonomy.	*Yes*
The program should promote equity and access to screening for the entire target population.	*Not applicable*
<u>The test and the treatment</u>	
There should be an accepted treatment for patients with recognised disease.	Not certain
Facilities for diagnosis and treatment should be available.	Yes
There should be a suitable test or examination.	Not certain
The test should be acceptable to the population.	Yes
There should be quality assurance, with mechanisms to minimise potential risks of screening.	*Not certain*
<u>The costs</u>	
The costs of case finding should be economically balanced in relation to possible expenditure on medical care as a whole.	Not certain
<u>Overall</u>	
There should be scientific evidence of screening program effectiveness.	*No*
The program should integrate education, testing, clinical services and program management.	*Not applicable*
Program evaluation should be planned from the outset.	*Not applicable*
The overall benefits of screening should outweigh the harm.	*Not certain*

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Chapter 4

Cost-effectiveness of a screening strategy for Q fever among pregnant women in risk areas: a clustered randomised controlled trial (study protocol)

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ABSTRACT

Introduction In The Netherlands the largest human Q fever outbreak ever reported in the literature is currently ongoing with more than 2300 notified cases in 2009. Pregnant women are particularly at risk as Q fever during pregnancy may cause maternal and obstetric complications. Since the majority of infected pregnant women are asymptomatic, a screening strategy might be of great value to reduce Q fever related complications. We designed a trial to assess the (cost-)effectiveness of a screening program for Q fever in pregnant women living in risks areas in The Netherlands.

Methods/design We will conduct a clustered randomised controlled trial in which primary care midwife centres in Q fever risk areas are randomised to recruit pregnant women for either the control group or the intervention group. In both groups a blood sample is taken around 20 weeks postmenstrual age. In the intervention group, this sample is immediately analysed by indirect immunofluorescence assay for detection of IgG and IgM antibodies using a sensitive cut-off level of 1:32. In case of an active Q fever infection, antibiotic treatment is recommended and serological follow up is performed. In the control group, serum is frozen for analysis after delivery. The primary endpoint is a maternal (chronic Q fever or reactivation) or obstetric complication (low birth weight, preterm delivery or foetal death) in Q fever positive women. Secondary aims pertain to the course of infection in pregnant women, diagnostic accuracy of laboratory tests used for screening, histo-pathological abnormalities of the placenta of Q fever positive women, side effects of therapy, and costs. The analysis will be according to the intention-to-screen principle, and cost-effectiveness analysis will be performed by comparing the direct and indirect costs between the intervention and control group.

Discussion With this study we aim to provide insight into the balance of risks of undetected and detected Q fever during pregnancy.

Trial registration ClinicalTrials.gov, protocol record NL30340.042.09.

INTRODUCTION

Q fever, a zoonosis caused by *Coxiella burnetii* (*C. burnetii*), primarily infects ruminants and rodents.[1] Especially pregnancy products of infected animals like placentas and amniotic fluid can contain high numbers of bacteria. After drying, the organism spreads in aerosols and remains virulent for months. Humans are infected by inhalation of these contaminated aerosols. Most of the infected patients are either asymptomatic or present with a mild flu-like illness. However, Q fever may pose a serious threat to certain groups at risk, including pregnant women, immune compromised hosts and individuals with pre-existing cardiac valve or vascular defects.[1,2] In The Netherlands, the number of human cases of Q fever has dramatically increased from around 12 cases each year before 2007 to more than 2300 cases in 2009.[3-5] This observation has led to several meetings of the Dutch Outbreak Management Team (OMT) of the Ministry of Health to curb the epidemic. Studies revealed that the epidemic among Dutch inhabitants was a result of Q fever outbreaks on dairy goat farms.[6]

Pregnant women are by far the largest risk group in size. When infected by *C. burnetii*, most pregnant women will remain asymptomatic: percentages up to 90% have been described compared to 60% in the general population.[7,8] Notably, serious complications due to Q fever seem to occur more frequently during pregnancy if the infection is undetected and untreated. Pregnant women have an increased risk to develop chronic Q fever or to reactivate a past infection.[9,10] Furthermore, obstetric complications related to *C. burnetii* infection have been described. A landmark study from France showed obstetric complications including spontaneous abortion, preterm delivery, intrauterine growth restriction, oligohydramnios and foetal death in 81% of the 53 women who were positive for Q fever and not sufficiently treated with antibiotics.[10] However, because of the retrospective design selection bias might have led to overestimation of the complication prevalence. In a Canadian cohort study in an affected area, 3.8% of parturient women had evidence of previous exposure to *C. burnetii*. These women had higher risks for adverse obstetrical outcome in terms of premature delivery and prior or current neonatal death.[11] Little is known about the chances of vertical transmission from mother to child. Transmission across the placenta, transmission by inhalation of infected amniotic fluid or by ingestion of infected milk cannot be excluded.

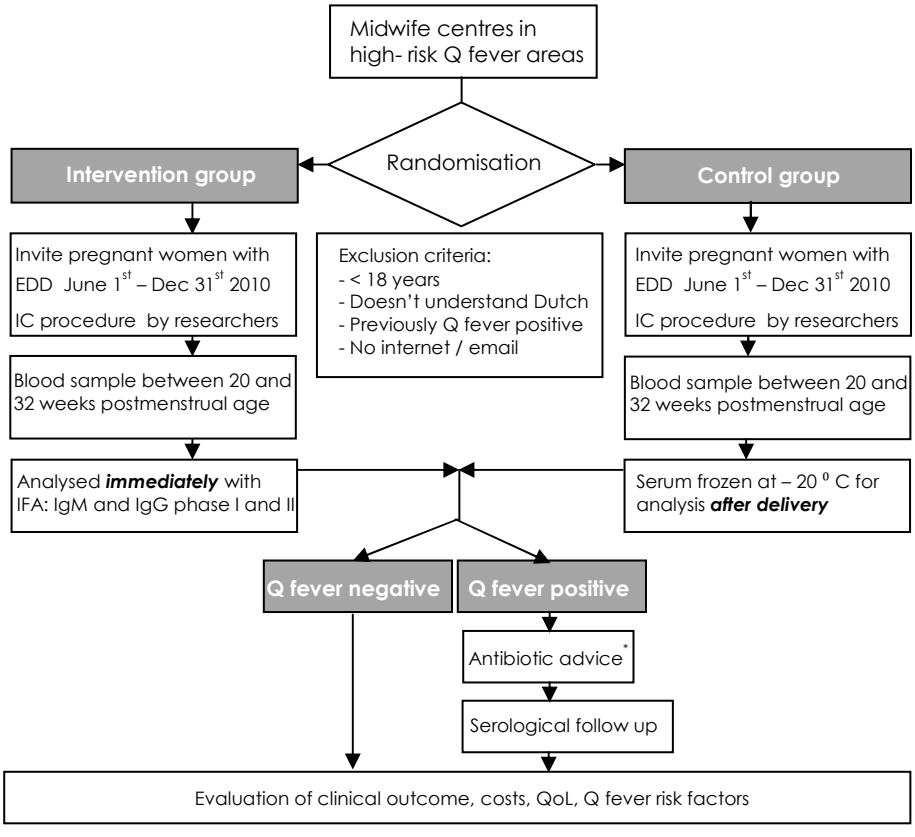
Because most infected pregnant women remain asymptomatic, one of the suggested measures to prevent obstetric complications and maternal chronicity concerns a screening strategy. However, because of lack of information on the prevalence during pregnancy and lack of randomised controlled trials weighing potential benefits and risks associated with screening, evidence for its potential impact is scarce. The Health Council of The Netherlands therefore advised the Ministry of Health in 2008 to facilitate studies to inform decision makers. We therefore designed a trial to assess the effects of a screening policy for Q fever in pregnant women from areas with large numbers of Q fever cases on the pregnancy outcome and cost-effectiveness from a societal and health care perspective. The study will primarily provide insights into the balance of risks of undetected and detected Q fever during pregnancy.

METHODS/DESIGN

Since ethical issues surrounding randomisation of the individual pregnant woman for a Q fever screening or non-screening strategy could seriously threaten approval by an ethics committee, we designed a clustered randomised controlled trial in which primary care midwife centres are randomised to recruit either pregnant women for the control group or for the intervention group. In this way, the choice for either strategy by individual eligible women was avoided. Timing and phasing after eligibility checks are shown in Figure 1. The study will be conducted according to the principles of the Declaration of Helsinki. The study protocol is approved by the Medical Ethical Review Board of the University Medical Center Groningen. The study protocol is registered at http://ClinicalTrials.gov, protocol record NL30340.042.09. The inclusion of participants started in April 2010.

The conduct of the trial is currently supported by the Royal Dutch Society for Midwifery (KNOV), the professional organisation of midwives. Midwife centres in risk areas for Q fever (incidence in 2009 of more than 50:100,000 inhabitants according to the National Institute for Public Health and the Environment (RIVM)), were primarily invited to facilitate inclusion of participants. During spring 2010 we expanded the area based on the incidence of 2010. All obstetricians, paediatricians, medical microbiologists and pathologists in these areas were informed about the study.

Figure 1. Timing and phasing of the study.



EDD, estimated date of delivery; IC, informed consent; IFA, indirect immunofluorescence assay; QoL, Quality of life. *Antibiotic treatment will be given according to the local hospital protocol. First choice treatment during pregnancy consists of cotrimoxazole 160/800mg b.i.d. for at least 5 weeks. After pregnancy doxycycline 100mg b.i.d. for at least two weeks is the preferred treatment for an active Q fever infection.

Inclusion criteria

Pregnant women, 18 years of age or older, with an estimated date of delivery between June 1st and December 31st 2010, and under supervision of a midwife in primary health care are eligible for inclusion. In The Netherlands, midwives working in primary health care are allowed to only supervise uncomplicated, singleton pregnancies. It is estimated that approximately 10,000 eligible pregnant women live in the Q fever affected areas.

Exclusion criteria

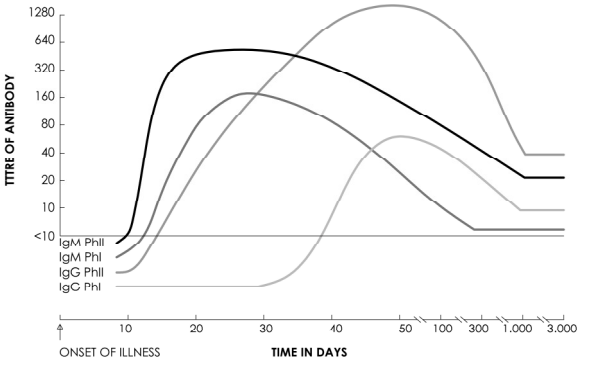
Women who do not have access to internet and/or an email address are excluded because data collection is web-based. In addition, women who are unable to understand Dutch or to give informed consent, or who have previously been tested positive for Q fever are ineligible for participation into the study.

Experimental procedure

Intervention group

Participants who are recruited by a midwife centre randomised for the intervention group are asked for a blood sample around 20 weeks postmenstrual age. If possible the visit is combined with the routine structural ultrasound scan around that time. If the participant is included after 20 weeks, the blood sample will be taken as soon as possible after inclusion. The sample will immediately be tested for antibodies against *C. burnetii* in the laboratory of the Jeroen Bosch Hospital which has analysed most samples during the epidemic in 2007, 2008, and 2009. Serologic diagnosis of Q fever will be made by indirect immunofluorescence assay (IFA), the reference method for serodiagnosis of Q fever.[12] Both IgM and IgG antibodies against phase I and phase II antigens are measured according to the manufacturer's instructions (Focus Diagnostics, Cypress, CA, USA). Titres ≥ 1:32 are considered positive. All positive samples will be fully titrated to reduce the chance of treatment in false positives. In general, the first antibody to appear in acute Q fever patients is IgM phase II, followed by a more or less simultaneous IgG phase II and IgM phase I response and subsequent appearance of IgG phase I antibodies (see Fig. 2).[13] This time-dependent serologic profile allows us to discriminate between a recent acute infection, a past infection, and a chronic infection.

Figure 2. Idealised antibody responses in acute Q fever as measured by IFA.[13]



If the pregnant woman does not have evidence for an acute, past or chronic Q fever infection, standard care will be provided. In case of acute Q fever, the participant will be referred to an obstetrician, and treatment will be advised according to the local hospital protocol. In the literature, the first choice treatment of Q fever during pregnancy is oral cotrimoxazole (sulfamethoxazole /trimethoprim) for at least 5 weeks.[10] Antibiotic treatment, further obstetrical care and serological follow up will be supervised by the obstetrician in collaboration with the medical microbiologist. The current routine for pregnant women being treated for acute Q fever is to perform monthly blood analyses to detect the development of chronic Q fever. If the titres decline, the frequency of these controls is scaled down to once every two months during pregnancy, and at 3, 6 and 12 months after delivery. If chronic Q fever develops, treatment will be continued until the end of pregnancy followed by bactericidal treatment with doxycycline and hydroxychloroquine after delivery. In Q fever cases placentas will be collected for polymerase chain reaction (PCR) and histo-pathology. If there is evidence for a past infection, no treatment is started. However, midwives will be advised to perform an extra serological analysis later in pregnancy to exclude reactivation.

Control group

Participants who are recruited by a midwife centre randomly allocated to the control group will also be asked for a blood sample around 20 weeks postmenstrual age. These blood samples will be stored at -20°C, and analysed for *C. burnetii* after delivery. In case of a positive test, the participant's general practitioner will be advised to perform an extra serological analysis to exclude chronic Q fever. Antibiotic treatment will be started if needed according to the local protocol.

Neonates

All neonates born to Q fever positive mothers will receive care according to the local hospital protocols. The Section for Paediatric Infectious Diseases and Immunology of the Dutch Paediatric Society has formulated a consensus guideline for neonates born to Q fever positive women during pregnancy.[14] The guideline advices PCR at birth and one month of age, and serological follow up until 18 months of age in case of active maternal Q fever during pregnancy to diagnose and treat potential mother-to-child transmission. Preventive antibiotic treatment is not advised. Breastfeeding is contraindicated if maternal serum or milk is *C. burnetii* PCR positive. Breastfeeding might also be contraindicated in case of maternal medication use.

Randomisation procedure

Participating midwife centres are randomised to include either pregnant women for the control group or for the intervention group. Randomisation is stratified according to the risk of contracting a *C. burnetii* infection as determined by the number of goat farms in the neighborhood (registration by Statistics Netherlands (CBS)), and by the size of the midwife centre.

Inclusion of participants

Pregnant women are invited by their midwife to participate in the study. The informed consent procedure will be performed by the researchers.

Data collection

Data will be collected in four ways using a structured case record file:

1. Serological samples will be collected at the time points described in the section Experimental procedure, and will be analysed in the laboratory of the Jeroen Bosch Hospital.
2. Questionnaires; two questionnaires will be filled out by the participant and one will be filled out by the midwife/obstetrician.
   At baseline, when the participant is included in the trial, a questionnaire is completed by all participants including questions about the current pregnancy, outcome of previous pregnancies, smoking and alcohol habits, co-morbidities, medication use and demographic characteristics. With this questionnaire risk factors are assessed for complicated pregnancy outcome. After delivery all relevant outcome data on obstetric complications are collected by a questionnaire completed by the midwife. Questionnaires for participants who are referred to a hospital during pregnancy or delivery, are filled out by the obstetrician. During follow up, all health care and potential cost data will be measured by a third questionnaire completed by the participant one month after delivery. With this questionnaire we will also verify symptoms during pregnancy, health-related quality-of-life (using EQ5D[15]), depressive symptoms and fatigue (using the Shortened Fatigue Questionnaire[16]), potential long-term consequences of Q fever, tolerance to antibiotic treatment and problems and development of the newborn. Furthermore, the risk for Q fever infection will be assessed.
3. PCR and histo-pathology of the placenta will be performed by the local microbiologists and pathologists. Re-evaluation of the histological slides will be performed by one pathologist at the University Medical Center Groningen.

4. Medical data in primary care; data on the health status of the participant or the newborn is collected from medical files of the general practitioner.

Outcome measures

The primary endpoint is a maternal (chronic Q fever or reactivation) or obstetric complication (low birth weight, preterm delivery or foetal death) after the first trimester of pregnancy in Q fever positive women.

The secondary endpoints are direct and indirect costs of the screening program compared to costs of complications which could be prevented by screening. Furthermore, we aim to assess the course of infection in pregnant women, the accuracy of the diagnostic tests used for screening, histo-pathological abnormalities of the placenta of Q fever infected women, and side effects associated with treatment.

Withdrawal of individual participants

Participants are informed that they can withdraw from the study at any time point, without giving a reason for withdrawal. If the blood sample has already been taken, participants will be asked to give permission for collecting data on obstetric outcome. Participants who withdraw will receive regular health care according to the local protocols.

Sample size calculation and statistics

Based on the literature and pilot data from The Netherlands, we expect that 12% of pregnant women in the high-risk areas will have serological evidence for a Q fever infection.[17,18] Of these women, we conservatively estimate that 25% will develop complications, so 3% of women will have the primary outcome. Assuming a reduction of the complication rate of 50% by early detection with screening during pregnancy, we will need a participation of at least 3400 participants with complete follow up (statistical power of 80 percent, $P \leq 0.05$). Assuming a loss to follow up of 10% and to allow for a small clustering effect, we aim to include 4000 participants. Data will be analysed according to intention-to-screen principles. A two-sided *P*-value of 0.05 or less will be considered to indicate statistically significant. Descriptive statistics concerning the distributions of the predictor variables and outcome variables will be performed using the software SPSS for windows (version 16). For univariate analysis the chi-square test and Fischer's exact test will be used to compare proportions. For variables with a normal distribution, differences will be analysed with Student's t-test. In case of non-parametric distribution, differences between populations will either be evaluated using the Mann-

Whitney-U test or the data will be log-transformed to obtain a normal distribution. Relative risks as well as absolute risk reductions and numbers needed to treat will be estimated with their corresponding 95% confidence intervals. Possible clustering of outcome data will be taken into account using generalised estimating equations (GEE) modelling.

Economic evaluation

The study will primarily provide insights into the economical balance of undetected and detected Q fever during pregnancy. The economic evaluation will be performed from a societal and health care perspective. Direct medical and non-medical costs (laboratory costs, costs of health care following positive screening, time, and travel costs) as well as indirect costs (loss of productivity) will be taken into account. The time horizon will be from taking the blood sample until one month after delivery for measured and calculated costs and until one year after delivery for estimated costs. Data on health care use and productivity loss will be collected by questionnaires. Unit costs will be based on the Dutch 2004 guidelines for costing in health care research and indexed for base year 2010 using yearly general consumer price indices.[19]

DISCUSSION

Right at the beginning of the first Q fever outbreak in The Netherlands in 2007, the government and health care providers assessed the risk of Q fever related to the outcome of pregnancies.[20] In all, only research from France is currently available on the risks of Q fever during pregnancy and the benefits of long-term antibiotic treatment.[10] There is, however, a lack of data on the prevalence and the risk of Q fever infection and the impact of antibiotic treatment during pregnancy in other countries such as The Netherlands. Therefore, in December 2008 the Dutch Health Council advised the Ministry of Health not to screen for Q fever during pregnancy until additional scientific data would be available to support screening.[20] The Dutch outbreak is an opportunity to gain more knowledge in this field. Therefore we will conduct the study described previously, to provide insights into the balance of risks of undetected and detected Q fever during pregnancy. By the end of February 2011 all data will be available for analysis. First results are expected in spring 2011.

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Chapter 5

Routine screening for *Coxiella burnetii* infection during pregnancy: a clustered randomised controlled trial

Submitted

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ABSTRACT

Background Since asymptomatic *Coxiella burnetii* infection has been associated with maternal and obstetric complications and outbreaks of human Q fever are reported more often in Europe since 2007, evidence about the effectiveness of routine screening for *Coxiella burnetii* infection during pregnancy in Q fever high-risk areas is needed.

Methods During the recent Dutch Q fever outbreak we performed a clustered randomised controlled trial in which 55 midwife centres were randomised to recruit pregnant women for an intervention or control strategy. In both groups a serum sample was taken between 20 and 32 weeks of gestation. In the intervention group (n=536) the samples were analysed immediately by indirect immunofluorescence assay and antibiotic treatment was given during pregnancy in case of an acute or chronic infection. In the control group (n=693), sera were frozen for analysis after delivery. For all participants data on pregnancy outcome were collected.

Results In both groups 15% of the women were seropositive. In the intervention group 2.2% of the women were seropositive and had an obstetric complication, compared with 1.4% in the control group (odds ratio 1.54 (95% confidence interval 0.60-3.96)).

Conclusion Routine *Coxiella burnetii* screening during pregnancy starting at 20 weeks of gestation seems not to be associated with a relevant reduction in obstetric complications.

Trial registration: ClinicalTrials.gov, identifier NCT01095328 [http://clinicaltrials.gov/ct2/show/NCT01095328?term=q+fever+pregnancy&rank=1]

WHAT IS ALREADY KNOWN ON THIS TOPIC?

Several outbreaks of human Q fever have taken place in Europe since 2007.

Pregnant women with a *Coxiella burnetii* infection are more often asymptomatic than the general population.

Undetected and untreated *Coxiella burnetii* infection during pregnancy was reported to be associated with both maternal complications (mainly chronic Q fever) and obstetric complications (miscarriage, preterm delivery, a child small for gestational age and foetal death).

WHAT THIS STUDY ADDS?

Fifteen percent of pregnant women living in Q fever high risk areas in The Netherlands have serological evidence for a previous or acute *Coxiella burnetii* infection.

Asymptomatic seropositivity is not associated with adverse pregnancy outcome.

Routine *Coxiella burnetii* screening during pregnancy starting at 20 weeks of gestation seems not to be associated with a relevant reduction in obstetric complications.

INTRODUCTION

Infections such as human immunodeficiency virus, syphilis and hepatitis B virus during pregnancy cause a threat to both maternal and foetal health, even if the infection is asymptomatic. Routine screening for infectious diseases is therefore recommended for millions of pregnant women worldwide.[1] Due to several outbreaks the incidence of Q fever, a zoonosis caused by the bacterium *Coxiella burnetii*, has been increasing in The Netherlands and other European countries since 2007.[2,3] Most of the infected individuals are either asymptomatic or present with a mild flu-like illness. However, *C. burnetii* may pose a serious threat to pregnant women because of the increased risk of chronic Q fever which is often complicated by endocarditis.[4-6] In addition, both symptomatic and asymptomatic *C. burnetii* infection during pregnancy have been associated with obstetric complications due to placentitis, including preterm delivery, intrauterine growth restriction and foetal death.[7,8] Because most infected pregnant women remain asymptomatic[9], routine serological screening could be of great value to prevent chronic maternal infections and obstetric complications, but evidence from randomised trials is lacking. Since the Dutch Q fever outbreak was with over 3500 cases in three years of time unique in its size[10], we had the opportunity to perform this clustered randomised controlled trial (RCT). The objective of this study was to assess the effectiveness of large-scale routine serological screening for *C. burnetii* infection during pregnancy in Q fever high-risk areas.

METHODS

Design

We conducted a clustered RCT in which primary care midwife centres were randomised to recruit pregnant women either for the intervention or for the control group (Fig. 1). The study was conducted according to the principles of the Declaration of Helsinki, and the study protocol was approved by the Medical Ethical Review Board of the University Medical Center Groningen. All participants gave written informed consent.

Setting

The study was set in Q fever high-risk areas in The Netherlands defined as municipalities with a Q fever incidence of more than 50 cases per 100,000

inhabitants in 2009 or more than 20 cases per 100,000 inhabitants in 2010 according to the official Dutch surveillance data.[11]

Randomisation procedure

Participating midwife centres in these high-risk areas were randomly allocated (ratio 1:1) using a computer-generated list of random numbers, containing random block sizes of 4 and 6 prepared by an investigator with no clinical involvement in the trial. Randomisation was stratified according to the risk-factor associated with contracting a *C. burnetii* infection by the number of goat farms in the municipality (up to 7 or >7)[12] and by the size of the midwife centre (up to 300 or >300 pregnant women under care per year). Since this was an open-label study, midwives, other health care workers, participants and the researchers were aware of the strategy.

Participants

Inclusion criteria

Pregnant women, 18 years of age or older, with an estimated date of delivery between June 1st and December 31st 2010, who were under supervision of a midwife in primary health care were eligible for inclusion. In The Netherlands, midwives working in primary health care are only allowed to supervise low-risk, singleton pregnancies. Using this criterion, women with an increased risk for complicated pregnancy outcome on forehand (i.e. twin pregnancies or pregnant women with chronic illnesses) were excluded.

Exclusion criteria

Women who did not have access to Internet or an email address were excluded because data collection was web-based. In The Netherlands, only 9% of the households do not have Internet access.[13] These households mainly consist of elderly or singles, so very little exclusion from this restriction was expected. In addition, women who were unable to understand Dutch, unable to give informed consent, or were already known as being Q fever positive were ineligible for participation in the study.

Interventions

Intervention group

Participants in the intervention group were asked for a serum sample between 20 and 32 weeks of gestation. The samples were analysed immediately by indirect immunofluorescence assay (IFA) in the laboratory of the Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands. Both immunoglobulin (Ig)M and

IgG against phase I and phase II antigens (Nine Mile strain) were measured according to the manufacturer's instructions (Focus Diagnostics, Cypress, CA, USA). Each run included a positive and a negative control. In line with the cut-off values used in the clinical setting for the diagnosis of Q fever in symptomatic patients, titres ≥ 1:32 were considered positive.[14] Of every positive sample the titre was determined to reduce the chance of false positivity. A probable acute infection was defined as the presence of positive titres of IgM (phase I and/or II) in the first screening sample. A proven acute infection was defined as positive titres for IgM accompanied with (rising) titres of IgG phase I and/or II during follow-up, two to four weeks after the screening sample. A previous infection was defined as the presence of only IgG (phase I and/or II) in the screening sample. A chronic *C. burnetii* infection was defined as an antibody titre of IgG phase I ≥ 1:1024.[15]

In seronegative women standard care was provided. In case of a (probable) acute or chronic *C. burnetii* infection, women were referred to an obstetrician and intensified serological and obstetric follow-up according to the local hospital protocol was given. Antibiotic treatment (cotrimoxazole (960 mg b.i.d.) or erythromycin (500 mg b.i.d to q.i.d.) depending on the term of pregnancy, for at least 5 weeks) was started in collaboration with the local medical microbiologist in any case of a proven acute or chronic infection. In case of a previous infection, no treatment was started, but serological analysis was repeated in the third trimester of pregnancy to exclude reactivation as part of a chronic infection.

Control group

Women in the control group were also asked for a serum sample between 20 and 32 weeks of gestation. These samples were centrally stored in the laboratory of the Jeroen Bosch Hospital at -20^{0}C and were analysed for antibodies against *C. burnetii* after delivery similar to the intervention group. In this group, distinguishing a probable and proven acute infection was impossible since follow-up serology during pregnancy was not performed. In case of a positive test, the participant's general practitioner was advised to perform an extra serological analysis after delivery to exclude a chronic infection.

Both groups

In case of symptoms compatible with Q fever during pregnancy, all participants were free to visit a physician for regular diagnostics.

Outcome measures

The primary endpoint pertained to the individual level and was a composite measure of a maternal or obstetric complication in seropositive women. A maternal complication was defined as the development of a serological profile suggesting chronic infection (IgG phase I ≥ 1:1024[15]). Obstetric complications included preterm delivery (defined as delivery <37 weeks of gestation), a child small for gestational age (SGA, defined as birth weight <10th percentile[16]), and perinatal mortality (defined as foetal or neonatal death between 22 weeks of gestation and one week post partum). Secondary endpoints were the separate components of the composite measure and fatigue and quality of life one month post partum. Fatigue was assessed using the Shortened fatigue questionnaire.[17] Quality of life was assessed using the validated EQ5D questionnaire.[18]

Sample size calculation

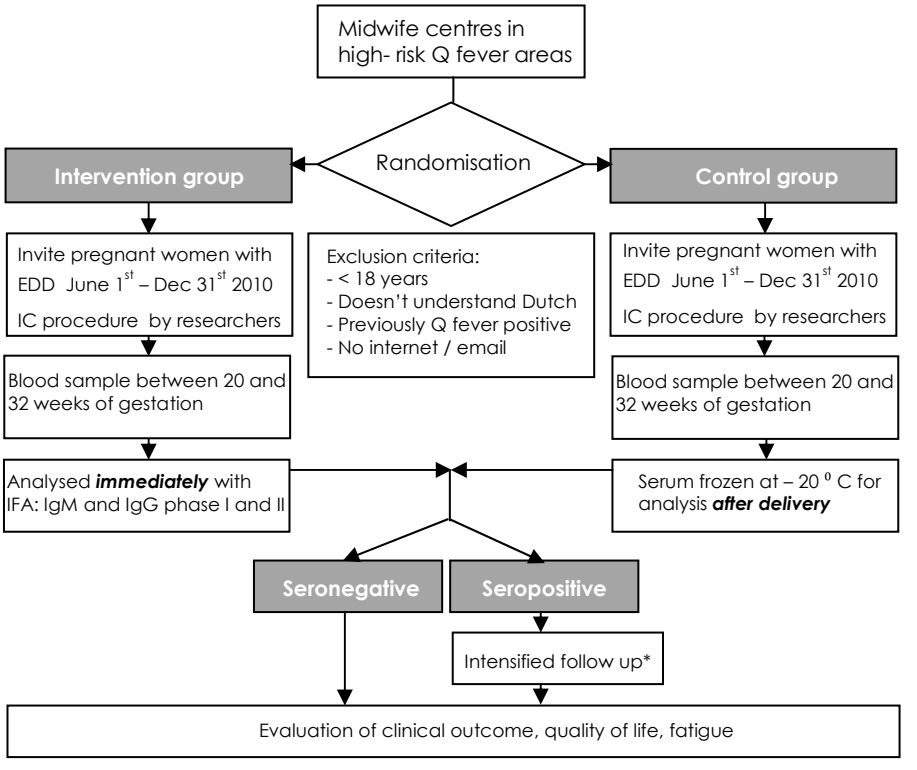
Since midwifery in primary health care is very much protocolised and serology was performed in one laboratory, the presence of clustering in the infrequent primary outcome of the study was expected to be minimal. Therefore the sample size calculation was performed on the individual level. Based on the literature and pilot data from The Netherlands, we expected that 12% of the pregnant women in the Q fever high-risk areas would be seropositive.[19,20] Of these women we estimated that at least 25% would have a complication. Thus, 3% of all pregnant women in Q fever high-risk areas would meet the primary outcome. A reduction of the complication rate by at least 50% as a consequence of early detection with screening during pregnancy was defined as clinically relevant. We considered reductions smaller than 50% unlikely to trigger a change in practice given the implications on health care resources. Based on these expectations, we estimated needing at least 3400 participants with complete follow-up (statistical power of 80%, a of 0.05).

Statistical methods

Data were analysed according to intention-to-screen principle. Baseline demographic information was summarised by group using frequencies with percentages for categorical variables and means with standard deviations for continuous variables. Odds ratio's (OR) and corresponding 95% confidence intervals (CI) were calculated using generalised linear mixed models (GLMM) to adjust for possible clustering effects. For continuous variables the mean difference with 95% CI was calculated. For the primary endpoint also the crude OR with 95% CI was calculated using binary logistic regression analysis, to

provide an indication of the extent of clustering. A two-sided *P*-value of 0.05 or less was defined as being statistically significant. Statistical analyses were performed using R version 12.1 and PASW Statistics version 18.0 (SPSS inc. Chicago, Illinois, USA).

Figure 1. Flow chart of the study protocol



EDD, estimated date of delivery; IC, informed consent; IFA, indirect immunofluorescence assay. * For the intervention group intensified serological follow-up and pregnancy monitoring with possible antibiotic treatment were performed during pregnancy under supervision of secondary health care. For the control group serological follow-up was performed after pregnancy in collaboration with the patients' general practitioner.

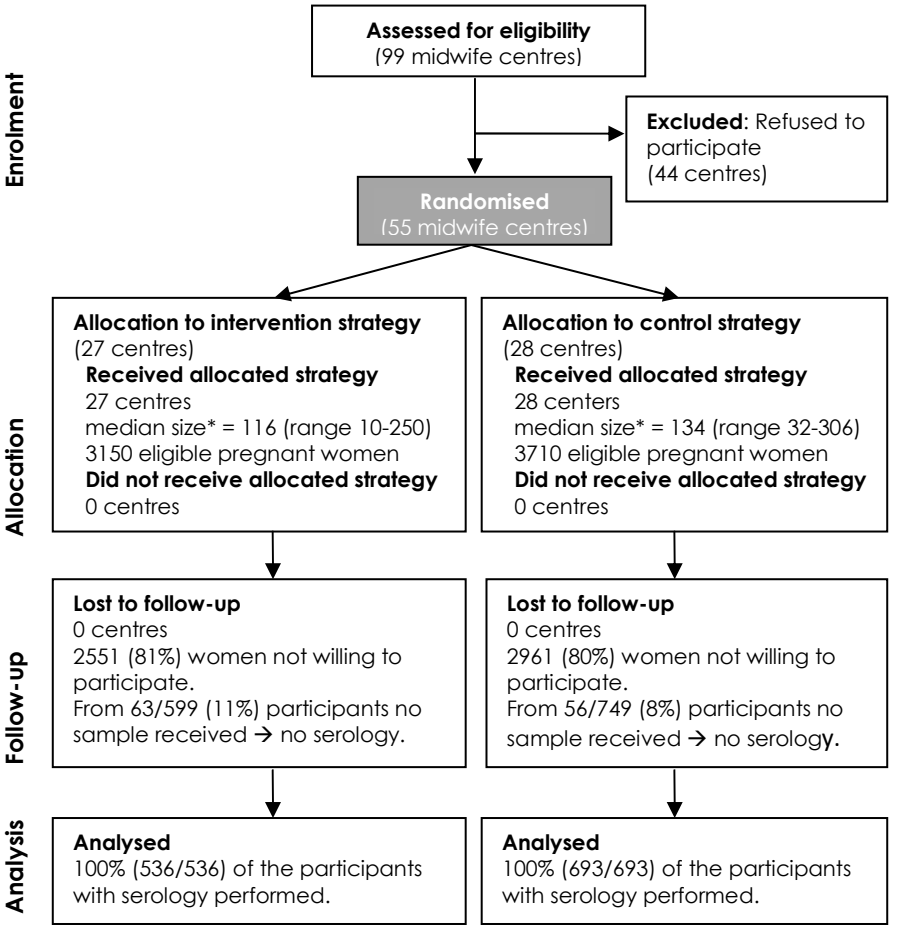
RESULTS

Between March 16 and July 17, 2010, 55 of the 99 eligible midwife centres were willing to participate and were randomised (27 to the intervention and 28 to the control strategy, Fig. 2). In total, these centres supervised 6860 eligible pregnant women of whom 1348 (20%) signed informed consent. Among these women a blood sample was collected for 1229 participants (536 participants in the intervention group and 693 in the control group). At the moment of screening, none of the participants suffered from a pneumonia or hepatitis (clinical signs of symptomatic Q fever[4]). Of 119 participants no blood sample was received, either because they forgot to give a sample or the sample was lost. These women were excluded from the analysis since the primary outcome measure was missing. Of 104 participants in the intervention group and 196 participants in the control group, the sample was taken outside the protocol period, i.e. before 20 weeks of gestation (n=7 and n=5, respectively) or after 32 weeks of gestation (n=97 and n=191). However, there was no difference in the baseline and outcome variables between the participants with and without this protocol deviation (data available on request), hence they were included in the analysis.

Baseline characteristics

Baseline characteristics are shown in Table 1. The mean gestational age at the moment of sampling was 28.7 weeks for the intervention group and 29.9 weeks for the control group. Fifteen percent of the women in both groups were seropositive for *C. burnetii*. Fifty-two of the 1229 participants had a probable acute infection (n=30 (5.6%) in the intervention group and n=22 (3.2%) in the control group) and 131 participants had a previous infection (n=52 (9.7%) in the intervention group and n=79 (11.4%) in the control group) according to the first screening sample. After follow-up seven women in the intervention group (1.3%) were diagnosed to have an acute *C. burnetii* infection and antibiotic treatment was started at a median stage of pregnancy of 28 weeks (range 22-36 weeks) for a duration of 1-5 weeks, depending on the serological follow-up and term of pregnancy. In the other patients with a probable acute infection (77%, 23/30) follow-up serology ruled out this suspicion and was consistent with a previous infection. Follow-up showed no cases of maternal chronic infections in either of the two groups, so only obstetric complications in seropositive women were recorded as an endpoint. None of the women in the intervention or control group were treated with antibiotics during pregnancy for symptomatic Q fever.

Figure 2. Flow chart of the progress of clusters and participants



***** Size of the midwife centre according to the number of eligible pregnant women under care.

Primary endpoint

For all the participants the primary outcome measure was available. There was no difference in the primary endpoint between the intervention and the control group (Table 2). The risk estimate obtained from the clustered analysis for an obstetric complication in seropositive women in the intervention group compared with the control group was 1.54 (95%CI 0.60-3.96). The un-clustered analysis showed a similar OR of 1.56 (95%CI 0.67-3.65). There were six cases of perinatal mortality (foetal death n=4, early neonatal mortality n=2). All these patients were seronegative.

Secondary endpoints

Analyses of the separate components of the composite measure showed that the difference in the primary endpoint in favour of the control group, though non-significant, seemed to be the result of a small difference in the risk of preterm delivery (Table 2).

The fatigue score one month post partum was approximately 1 point higher in the intervention group compared with the control group (14.6 versus 13.5, $P<0.001$). Quality of life did not differ between the two groups. (Table 3)

Explorative analysis showed that *C. burnetii* seropositivity during pregnancy, even when the cut-off titre for seropositivity was increased to ≥ 1:64, was not associated with gestational age at delivery, birth weight or any of the defined obstetric complications (Table 4). From the seven women in the intervention group with an acute infection two women delivered preterm and one woman delivered from a child small for gestational age.

DISCUSSION

We showed that large-scale routine serological screening for *C. burnetii* infection during pregnancy starting at 20 weeks of gestation in Q fever high-risk areas seems not to be associated with a relevant reduction in obstetric complications in seropositive women. Therefore, our data do not support such a preventive program. This finding can be explained by the low incidence of acute *C. burnetii* infection (1.3%), the absence of patients with a chronic infection and the finding that *C. burnetii* seropositivity was not associated with adverse pregnancy outcomes. Surprisingly, we observed that participants of the intervention group had a somewhat higher fatigue score one month post partum than controls. Although the clinical relevance may be questionable, other screening strategies for infectious diseases during pregnancy have shown that screening for and therefore awareness of infectious diseases may induce negative psychological effects.[21]

Importantly, despite the fact that this study was performed in a Q fever high-risk area and participation of midwife centres was high (56%), the participation rate of pregnant women was unexpectedly low (20%). Although it's likely that this low percentage reflects a reluctance to take part in a randomised controlled trial, this might also indicate that the acceptance of such a preventive program among this group might not be straightforward.

Strengths and limitations

A major strength of this study is that it is the first randomised, prospective, study in a community based - non-selected - pregnant population focusing on the effectiveness of routine screening for *C. burnetii* infection. Since the Dutch Q fever outbreak between 2007 and 2010 was unique in its magnitude and duration, we had the opportunity to perform this study in a high-risk area. However, probably due to the drastic veterinary measures taken by the Dutch government the incidence of acute *C. burnetii* infections steeply declined since 2010.[10] Inclusion of participants after the second half of 2010 would not have been informative and was perceived as unethical. Therefore, we did not reach our projected number of inclusions, which increases the risk of a type II error. However, this risk seems to be minimal, because the lower estimate of the 95% confidence interval of the primary outcome (OR 0.60) precludes the a priori defined 50% risk reduction in relevant outcomes.

There are also some other limitations to address. In this study screening started at 20 weeks of gestation. There are two main reasons why we chose for this design. First of all we aimed to avoid treatment with a drug (cotrimoxazole) that is not completely investigated in pregnancy, during the most vulnerable phase of pregnancy.[22] Earlier screening and withholding treatment till 20 weeks of gestation was perceived as unethical and therefore not an option. Secondly, at 20 weeks of gestation pregnant women could combine the venepuncture for this study with a structural ultrasound, which is offered to all pregnant women in the Netherlands; a method to increase the participation rate. Because of this design screening in the first trimester of pregnancy is still untested, and effectiveness of such a strategy can not be excluded. However, a recent Danish study showed no association between *C. burnetii* infection and miscarriage up to 22 weeks of gestation[23], indicating that screening earlier in pregnancy would probably also be ineffective.

Furthermore, 44% of the eligible midwife centres and 80% of the eligible pregnant women were not willing to participate, so generalisability might be at stake. However, since major patient characteristics like maternal age and proportion of nulliparous women are comparable with other large population based cohort studies from The Netherlands we believe our results are applicable to this setting.[24,25] Nevertheless pregnant women with a non-Western ethnicity were underrepresented in our study population so our results should be interpreted with caution for this group, especially because it is known that the seroprevalence in pregnant women with a non-Dutch ethnic background is higher.[26]

Serological screening during pregnancy is challenging. A high rate of false-positivity has been described, especially for IgM assays.[27,28] Furthermore, the specificity of tests may be low if the incidence of the disease is relatively low and the prevalence is relatively high. Of every positive sample the titre was determined and we performed serological follow-up of all IgM positive women to prevent treatment of false-positive acute cases.

Comparisons with other studies

In contrast to our results, previous studies reported a strong association between undetected and untreated *C. burnetii* infection during pregnancy and complicated pregnancy outcome.[7,8,29] One explanation might be that in the previous non-randomised studies, selection bias could have led to an overestimation of the risks. Otherwise, differences in pathogenicity between different *C. burnetii* strains could exist. Genotyping of Dutch samples is ongoing.[30] Since in The Netherlands a relatively high number of chronic Q fever has been described in patients with aneurysms[31], it could be hypothesised that the strains involved in the Dutch outbreak are highly virulent for people with underlying vascular diseases, while pregnant women are relatively protected.

There are also studies in line with our results. In two large studies conducted in Q fever high-risk areas in The Netherlands and France an association between seropositivity and complicated pregnancy outcome was not found.[26,32]

CONCLUSION

This clustered randomised controlled trial showed that 15% of the pregnant women in Q fever high-risk areas are seropositive, but the incidence of acute *C. burnetii* infection is low. Although the broad confidence interval did not exclude a small beneficial effect of screening, routine screening during pregnancy starting at 20 weeks of gestation seems in any case not to be associated with a *relevant* reduction of obstetric complications in seropositive women. Therefore, in the current setting, this study does not support such a preventive program.

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Table 1. Baseline characteristics of the clusters and participants

	Intervention group	Control group
Midwife centre characteristics		
Numbers	27	28
Size:		
≤300 women per year	14 (52)	13 (46)
>300 women per year	13 (48)	15 (54)
Goat farms in municipality:		
≤7	13 (48)	14 (50)
>7	14 (52)	14 (50)
Participant characteristics		
Numbers	536	693
Age (in years)	31.9 ± 3.8	31.7 ± 3.7
Nulliparous	252 (47)	295 (43)
Ethnic origin non-Western	14 (2.6)	12 (1.7)
Education[a]		
Low	29 (5.4)	49 (7.1)
Medium	177 (33)	228 (33)
High	319 (60)	411 (59)
Other/Unknown	11 (2.1)	5 (0.7)
Maternal smoking during pregnancy	54 (10)	54 (7.8)
Body mass index (in kg/m^2)[b]	23.8 ± 3.7	24.1 ± 4.0
Primary hypertension	5 (0.9)	3 (0.4)
Hypothyroidism	6 (1.1)	11 (1.6)
History of preterm delivery	20 (3.7)	24 (3.5)
History of miscarriage[c]		
No	411 (77)	550 (79)
One	97 (18)	115 (17)
Repeated	27 (5.0)	27 (3.9)
Gestational age at the moment of sampling	28.7 ± 4.7	29.9 ± 4.8
Coxiella burnetii seropositive	82 (15)	101 (15)

Data are presented as mean ± standard deviation or n (%) of clusters or patients.

[a] Low represents: no education, primary school, lower-middle secondary school and lower professional school; medium represents: medium professional school and higher secondary school; high represents: higher professional school and university.

[b] Prior to pregnancy.

[c] n=535 for intervention group and n=692 for control group.

Table 2. Complications in seropositive participants

	Intervention group		Control group		Adjusted OR[a] [95% CI]	P[a]	Unadjusted OR[b] [95% CI]	P[b]
	Total n=536	Seropositives n=82	Total n=693	Seropositives n=101				
Overall complication[c]	12 (2.2)	12 (14.6)	10 (1.4)	10 (9.9)	1.54 [0.60-3.96]	0.37	1.56 [0.67-3.65]	0.30
Preterm delivery	8 (1.5)	8 (9.8)	5 (0.7)	5 (5.0)	1.80 [0.37-8.72]	0.47	2.09 [0.68-6.41]	0.20
Small for gestational age	4 (0.7)	4 (4.9)	5 (0.7)	5 (5.0)	1.04 [0.28-3.87]	0.96	1.04 [0.28-3.87]	0.96
Perinatal mortality	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	not applicable			

OR, odds ratio; CI, confidence interval. Data are presented as no. (%) or OR [95% CI]

[a] Odds ratio and *P*-value calculated with generalised linear mixed models, taking into account a clustering effect.

[b] Crude odds ratio and *P*-value calculated with binary logistic regression analysis.

[c] Primary outcome measure

Table 3. Fatigue and Quality of Life one month post partum

	Intervention group	Control group	OR[a] [95% CI]	Mean difference[a] [95% CI]	*P*[a]
Fatigue score[b]	14.6 ± 5.7	13.5 ± 5.5		1.08 [0.43-1.72]	<0.001
Quality of Life[c]					
Mobility ≥ 2	58 (12)	86 (14)	0.86 [0.60-1.23]		0.42
Self-care ≥ 2	3 (0.6)	3 (0.5)	1.31 [0.26-6.50]		0.75
Usual activities ≥ 2	74 (15)	99 (16)	0.97 [0.70-1.35]		0.85
Pain/discomfort ≥ 2	132 (27)	179 (28)	0.94 [0.72-1.24]		0.68
Anxiety/depression ≥ 2	27 (5.5)	38 (6.0)	0.92 [0.56-1.53]		0.75
EQ VAS[d]	80.1 ± 11.6	81.4 ± 12.1		1.18 [-0.39-2.75]	0.14

OR, odds ratio; CI, confidence interval. Data are presented as mean ± standard deviation, n (%) and OR [95% CI]

[a] Odds ratio, mean difference and *P*-value calculated with generalised linear mixed models, taking into account a clustering effect.

[b] n=506 and 662 for the intervention and control group, respectively. Range of the score 4 (not fatigue) to 28 (extreme fatigue).

[c] n=488 and 636 for the intervention and control group, respectively. A score of 1 resembles no problems, 2 corresponds with any problems and 3 with major problems.

[d] Self-reported health score on scale from 0 to 100, where a score of 100 represents the 'Best imaginable health state' and a score of 0 represents the 'Worst imaginable health state'. Cases with a score lower than 11 were excluded (n=30), since a mistake while filling out was assumed.

Table 4. Pregnancy outcome for seropositive[a] versus seronegative participants

	Seropositive n=183	Seronegative n=1046	OR[b] [95% CI]	Mean difference[b] [95% CI]	*P*[b]
Gestational age at delivery (in weeks)	39.6 ± 1.8	39.7 ± 1.7		0.12 [-0.15-0.38]	0.38
Preterm delivery <37 weeks	13 (7.1)	58 (5.5)	1.30 [0.70-2.43]		0.41
Very preterm delivery <34 weeks	3 (1.6)	13 (1.2)	1.32 [0.37-4.69]		0.66
Birth weight (in grams)	3512 ± 527	3507 ± 546		4.8 [-81-90]	0.91
Small for gestational age	9 (4.9)	78 (7.5)	0.64 [0.32-1.30]		0.22
Perinatal mortality	0 (0.0)	6 (0.6)	not applicable		0.60[c]
Overall complication[d]	22 (12)	133 (13)	0.94 [0.58-1.52]		0.79

OR, odds ratio; CI, confidence interval. Data are presented as mean ± standard deviation, no. (%) or OR [95% CI]

[a] Using a cut-off titre of ≥ 1:32

[b] Odds ratio, mean difference and *P*-value calculated with generalised linear mixed models, taking into account a clustering effect.

[c] Calculated with Fisher's exact test, since generalised linear mixed models could not provide a *P*-value.

[d] Composite measure of any preterm delivery, small for gestational age, or perinatal mortality.

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Chapter 6

The value of *Coxiella burnetii* serology in predicting adverse obstetric outcome in an endemic area

Submitted

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ABSTRACT

Objective *Coxiella burnetii* infection during pregnancy has been associated with adverse obstetric outcome. As part of a randomised controlled trial conducted in Q fever high-risk areas in The Netherlands we had the unique opportunity to study the predictive value of *C. burnetii* serological status in addition to well-known risk factors for obstetric complications.

Methods Pregnant women who were not treated with antibiotics for a *C. burnetii* infection were selected from the trial population. These women were under supervision of a midwife in primary health care and were followed prospectively from approximately 20 weeks of gestation on. We evaluated the capacity of maternal characteristics with or without *C. burnetii* serological status to predict adverse obstetric outcome. Adverse obstetric outcome was defined as any preterm delivery, child small for gestational age or perinatal mortality. The performance of the logistic regression models was assessed with receiver-operating-characteristic (ROC) analysis and calibration plots.

Results In all, of the 1221 included women, 152 (12.4%) had an adverse outcome. The prediction model including well-known risk factors such as smoking, nulliparity and low education-level was well calibrated, but had low predictive value (area under the ROC-curve 0.68; 95% confidence interval 0.63-0.72) with predicted rates of adverse outcome ranging from 4% to 27%. Addition of *C. burnetii* serological status to the model did not improve its predictive value.

Conclusions In a low-risk obstetric population from high-risk Q fever areas, prediction of adverse obstetric outcome is difficult and knowledge of *C. burnetii* serological status does not contribute to a better prediction.

INTRODUCTION

Adverse outcome of pregnancy in terms of preterm delivery, a child small for gestational age (SGA) or perinatal mortality is still a considerable problem even in developed countries.[1-3] Several conditions in the woman's medical history or during her pregnancy have been associated with these adverse outcomes including maternal infections.[4]

The incidence of Q fever, a zoonosis caused by the bacterium *Coxiella burnetii*, has dramatically increased in Europe since 2007.[5,6] If infected with the bacterium up to 90% of the pregnant women remain asymptomatic.[7] However, both symptomatic and asymptomatic infection during pregnancy have been associated with obstetric complications.[8-10] Since routine screening during pregnancy might prevent complications[4], we performed a clustered randomised controlled trial (RCT) showing that routine screening for Q fever was ineffective in reducing the risk of obstetric complications. Furthermore, *C. burnetii* seropositivity was not associated with obstetric complications in a univariable analysis.[11] However, it is questionable whether knowledge about seropositivity will contribute to a better prediction of adverse obstetric outcome in relation to other well-known risk factors. Prediction of adverse outcome in obstetrical care is desirable, especially in the unique Dutch obstetric care system, where before or at the beginning of pregnancy a distinction is made between women with a low risk of pathology (supervision of midwifes in primary care) and women with an increased risk (supervision of an obstetrician in secondary care).[12]

In the present study we assessed the additive predictive value of *C. burnetii* serological status in relation to other well-known risk factors for adverse obstetric outcome in a low-risk pregnant population living in an endemic Q fever area.

PARTICIPANTS AND METHODS

Setting and participants

We used data from the clustered RCT on screening for *C. burnetii* infection during pregnancy, which was conducted in The Netherlands between March 2010 and March 2011.[11] In short, 55 primary care midwife centres in endemic Q fever areas were randomly allocated to a screening or a control strategy. In both groups a blood sample was taken from pregnant women who gave informed consent, between 20 and 32 weeks of gestation. In the intervention

group samples were analysed immediately by indirect immunofluorescence assay (IFA) in the laboratory of the Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands. Both immunoglobulin (Ig)M and IgG antibodies against phase I and phase II antigens (Nine mile strain) were measured according to the manufacturer's instructions (Focus Diagnostics, Cypress, CA, USA). Each run included a positive and a negative control. In line with the cut-off values used in the clinical setting for the diagnosis of Q fever in symptomatic patients, titres ≥ 1:32 were considered positive.[13] In case of positive serology, intensified serological follow up with possible antibiotic treatment of patients with an active *C. burnetii* infection was performed. In the control group samples were frozen for analysis after delivery. The study was conducted according to the principles of the Declaration of Helsinki and the study protocol was approved by the Medical Ethical Review Board of the University Medical Center Groningen.

Only participants who were not treated with antibiotics for a *C. burnetii* infection were included in the current study. Participants in the intervention and control group were combined in one cohort.

Outcome measures and predictor selection

The primary endpoint was a composite measure of obstetric complications, which was defined as any preterm delivery, child small for gestational age (SGA) or perinatal mortality. Preterm delivery was defined as delivery before 37 weeks of gestation, SGA was defined as birth weight below the 10th percentile according to the birth weight curves of The Netherlands perinatal registry[14], and perinatal mortality was defined as foetal or neonatal death between 22 weeks of gestation and one week post partum.

We evaluated whether the composite endpoint could be predicted using well-known risk factors including patient's demographic characteristics (maternal age, non-Western ethnicity, low education-level, prepregnancy body mass index (BMI)), medical condition (primary hypertension, cardiac disease, hypothyroidism), obstetric history (nulliparity, history of miscarriage, history of preterm delivery, history of perinatal mortality), lifestyle factors (alcohol use and smoking during pregnancy), current clinical characteristics (gestational hypertension, gestational diabetes, vaginal blood loss in the second half of pregnancy, suspected foetal distress during pregnancy (mainly consisting of subjective maternal experience of less foetal movements), male gender of the child) and *C. burnetii* specific serological status. Marginal exploratory analysis was performed on the continuous variables maternal age and prepregnancy BMI to analyse whether these were best represented in the model as

continuous or categorical variables. Based on this analysis, maternal age was used as a continuous variable and BMI was categorised in < 25 percentile (BMI < 21.3 kg/m^2) and ≥ 25 percentile, of which the second category was the reference category.

Data analysis

Baseline demographic information was summarised by outcome group using frequencies and proportions for categorical variables and means and standard deviations for continuous variables. Binary logistic regression analysis was performed to predict the occurrence of the primary endpoint from patient characteristics and *C. burnetii* serological status in a univariable and multivariable approach. Only predictors with a frequency of five or higher per outcome-group were included in the multivariable analysis. Odds ratio's (OR) and corresponding 95% confidence intervals (CI) were calculated. Two methods were used to build the multivariable model. First we applied the full model approach, in which only well-known candidate risk factors were included in the model irrespective of their *P*-value, to avoid overfitting of the model[15]: the base model. Secondly, we added *C. burnetii* serological status, to determine the contribution of this variable. The performance of the logistic regression models was assessed by calibration and discrimination.[16] Calibration was evaluated by plotting the observed probabilities against deciles of the predictive probabilities and by the Hosmer-Lemeshow test, where a high *P*-value (*P*>0.05) indicates good calibration. The area under the receiver operating characteristic (ROC) curve was used to summarise the ability of the model to discriminate between individuals with and without the primary outcome event. The validity of the two models was evaluated by bootstrap analysis. Resampling with replacement from the original dataset was used to construct 200 bootstrap models. Calculations were performed using PASW Statistics version 18.0 (SPSS inc. Chicago, Illinois, USA) and graphs were produces using GraphPad Prism version 4.03 (GraphPad Software inc. La Jolla, CA, USA). Bootstrapping was performed with R version 2.13.0.

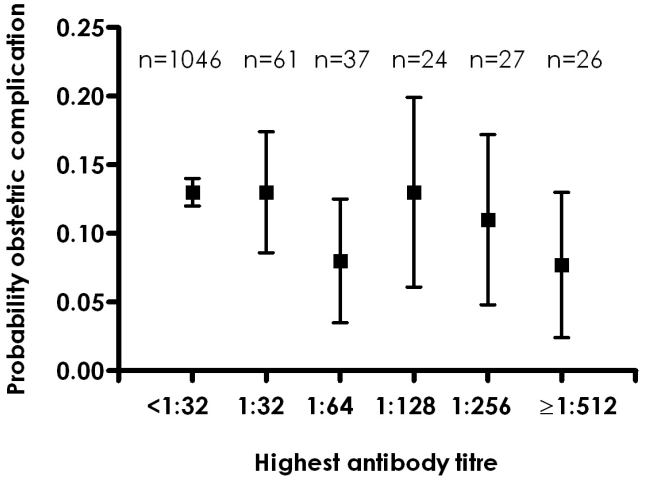
RESULTS

Of the 6860 eligible pregnant women of the clustered RCT 1348 signed informed consent. For this study, 127 participants were excluded because serology was not performed (n=119) or the participant was treated with

antibiotics for a *C. burnetii* infection (n=8). Baseline characteristics of the 1221 participants in relation to the primary endpoint are shown in Table 1.

The primary outcome measure was available for all participants. An obstetric complication occurred in 152 of the 1221 (12.4%) participants. Preterm delivery occurred in 69 (5.7%) participants, SGA in 86 (7.0%) and perinatal mortality in 6 (0.5%) women. Eight participants had more than one complication. In a univariable analysis participants with an obstetric complication were more often nulliparous (*P*<0.001), smoked cigarettes during pregnancy more often (*P*=0.004), had lower body mass index (*P*=0.001), gave more often birth to a boy (*P*=0.043) and their pregnancy was more often complicated with vaginal blood loss in the second half of pregnancy (*P*=0.011) and suspected foetal distress (*P*=0.040) (Table 1). In total, 175 of the 1221 participants (14.3%) were *C. burnetii* seropositive (only IgM n=5, only IgG n=131, both IgM and IgG antibodies n=39). The presence of *C. burnetii* antibodies was not associated with adverse obstetric outcome in both the univariable and multivariable model (OR 0.84, 95% CI 0.50-1.39, *P*=0.491 and OR 0.90, 95% CI 0.52-1.54, *P*=0.694, respectively). Also when the height of the antibody titres was taken into account there was no association with adverse obstetric outcome (Fig. 1).

Figure 1. Probability of obstetric complications in relation to the highest *C. burnetii* antibody titre



The probability of an obstetric omplication (mean with standard error) displayed by the highest antibody titre (IgM phase I or II, or IgG phase I or II). Obstetric complication is defined as any preterm delivery, SGA or perinatal mortality.

Table 1. Baseline characteristics and univariable analysis of the predictors in relation to obstetric complications.

	N	Obstetric complication[a] n=152, 12.4%	No obstetric complication n=1069, 87.6%	OR [95% CI]	*P*
Maternal age (mean with SD, in years)	1221	31.7 ± 3.7	31.8 ± 3.8	0.99 [0.95-1.04]	0.794
Nulliparous	1219	90 (59)	451 (42)	1.98 [1.40-2.80]	<0.001
Ethnic origin non-Western	1212	2 (1.3)	24 (2.3)	0.58 [0.14-2.48]	0.462
Low educated[b]	1211	13 (8.6)	64 (6.0)	1.47 [0.79-2.73]	0.228
Smoking during pregnancy	1215	23 (15)	84 (7.9)	2.08 [1.27-3.41]	0.004
Alcohol use during pregnancy	1215	1 (0.7)	24 (2.3)	0.29 [0.04-2.15]	0.225
BMI (kg/m^2) <25th percentile[c]	1210	55 (36)	246 (23)	1.89 [1.32-2.72]	0.001
Primary hypertension	1215	1 (0.7)	7 (0.7)	1.01 [0.12-8.24]	0.995
Hypothyroidism	1215	2 (1.3)	15 (1.4)	0.94 [0.21-4.15]	0.933
Cardiac disease	1215	1 (0.7)	5 (0.5)	1.41 [0.16-12.17]	0.754
History of perinatal mortality	1218	0 (0.0)	5 (0.5)	not applicable	
History of preterm delivery	1209	6 (3.9)	38 (3.6)	1.10 [0.46-2.65]	0.828
History of miscarriage	1219	41 (27)	224 (21)	1.39 [0.94-2.05]	0.096
Male gender child	1219	85 (56)	503 (47)	1.42 [1.01-2.00]	0.043
Gestational hypertensive disorder	1215	16 (11)	72 (6.8)	1.65 [0.93-2.92]	0.087
Gestational diabetes	1213	2 (1.3)	9 (0.8)	1.57 [0.34-7.34]	0.566
Vaginal blood loss 2nd half of pregnancy	1215	10 (6.6)	28 (2.6)	2.62 [1.25-5.52]	0.011
Suspected foetal distress during pregnancy	1216	11 (7.3)	39 (3.7)	2.07 [1.04-4.13]	0.040
Coxiella burnetii seropositive	1221	19 (13)	156 (15)	0.84 [0.50-1.39]	0.491

OR, odds ratio; CI, confidence interval; BMI, body mass index. Data are number (percentage) or odds ratio [95% confidence intervals] unless otherwise specified.

[a] Obstetric complication is defined as any preterm delivery, SGA or perinatal mortality

[b] Low represents: no education, primary school, lower-middle secondary school and lower professional school; non-low educated represents: medium professional school and higher secondary school, higher professional school and university. [c] Prior to pregnancy.

Ethnicity, alcohol use, primary hypertension, hypothyroidism, cardiac disease, history of perinatal mortality and gestational diabetes were not included in the multivariable model since their frequency was below five in de group with an obstetric complication. The results of the predictive value of the other variables in a multivariable setting are shown in Table 2.

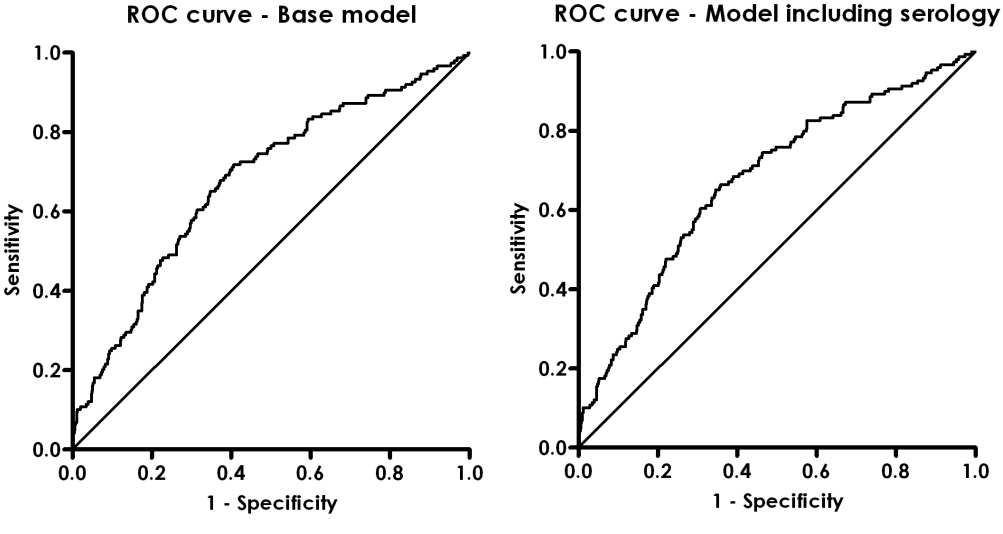
Table 2. Multivariable analysis of the predictors[a] in relation to adverse obstetric outcome

	Base model n=1194			**Base model including *C. burnetii* antibody status n=1194**		
	OR (95% CI)	**RC**	***P***	**OR (95% CI)**	**RC**	***P***
Maternal age (years)	1.03 (0.98-1.08)	0.030	0.239	1.03 (0.98-1.08)	0.030	0.239
Nulliparous	2.23 (1.50-3.32)	0.803	<0.001	2.23 (1.50-3.32)	0.801	<0.001
Low educated	1.33 (0.67-2.64)	0.285	0.414	1.34 (0.68-2.65)	0.291	0.405
Smoking during pregnancy	1.86 (1.08-3.22)	0.622	0.026	1.85 (1.07-3.20)	0.616	0.027
BMI (kg/m^2) <25th percentile[a]	1.91 (1.32-2.78)	0.648	0.001	1.90 (1.31-2.77)	0.643	0.001
History of preterm delivery	1.66 (0.66-4.15)	0.504	0.283	1.65 (0.66-4.15)	0.503	0.283
History of miscarriage	1.49 (0.98-2.27)	0.398	0.065	1.48 (0.97-2.26)	0.394	0.067
Male gender child	1.45 (1.02-2.08)	0.374	0.040	1.45 (1.02-2.07)	0.373	0.040
Gestational hypertensive disorder	1.54 (0.85-2.82)	0.434	0.157	1.55 (0.85-2.82)	0.436	0.155
Vaginal blood loss 2nd half of pregnancy	2.49 (1.15-5.37)	0.911	0.020	2.47 (1.14-5.34)	0.903	0.022
Suspected foetal distress during pregnancy	1.91 (0.93-3.90)	0.645	0.078	1.92 (0.94-3.92)	0.649	0.076
Coxiella burnetii seropositive				0.90 (0.52-1.54)	-0.108	0.694

RC, regression coefficient; BMI, body mass index. Data are odds ratio (95% confidence intervals). [a] Prior to pregnancy.

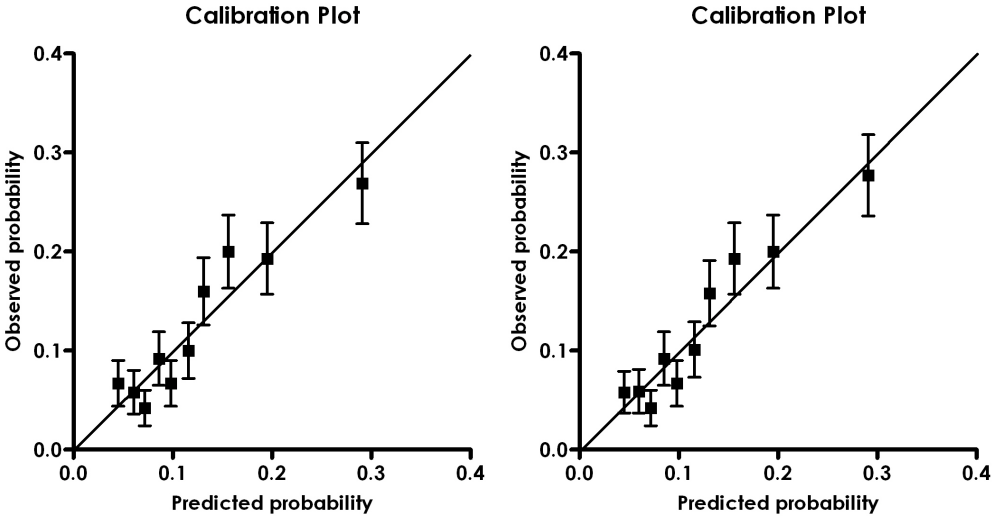
The base model gave a moderate discrimination, with an area under the ROC curve of 0.68 (95% CI 0.63-0.72, *P*<0.001) (Fig. 2a). Plotted calibration was moderate with predicted rates of obstetric complications ranging from 4% for the lowest decile to 27% for the highest decile (Fig. 3a), but the Hosmer-Lemeshow *P*-value was high (*P*=0.49). When we added *C. burnetii* serological status to the model, the area under the ROC curve and the calibration remained unchanged (AUC 0.68, 95% CI 0.63-0.72, *P*<0.001, and Hosmer-Lemeshow *P*=0.62) (Fig. 2b and 3b). In both models there was a small overestimation of the risk for an obstetric complication in the lowest risk groups (<12% predicted probability) and a small underestimation of the risk in the higher risk groups (13 to 16% predicted probability) (Fig. 3a and 3b). Bootstrapping showed for both models an AUC of 0.65, indicating good validity and absence of strong overfitting.

Figure 2. ROC curves of the base model (a) and the model including *Coxiella burnetii* serology (b), calculated by multivariable analysis



ROC, receiver-operating-characteristic
Area under the curve of both curves: 0.68 with confidence interval 0.63-0.72.

Figure 3. Calibration plot of the base model (a) and the model including *Coxiella burnetii* serology (b)



DISCUSSION

In a low-risk pregnant population from an endemic Q fever area, *C. burnetii* serological status was not of additive value in the prediction of obstetric complications. The AUC remained 0.68, which is moderate compared to other prediction models in obstetrics.[17,18] The reason for this low discriminative capacity could be the low-risk population, which we selected on forehand by including only women under supervision of a midwife in primary health care. Since women with important risk factors, e.g. twin-pregnancies or severe underlying morbidities, were therefore already excluded, only weaker risk-factors remained as input variables for the model. This also explains why the variability in predicted probabilities was moderate (4% for the lowest decile to 27% for the highest decile). In contrast to other studies, we did not identify *C. burnetii* seropositivity as a risk factor for obstetric complications. In a large Canadian cohort study, including 7658 parturient women, it was shown that IgG seropositivity in umbilical cord blood, which was the case in 3.8% of the women, was independently associated with preterm delivery and current and prior neonatal death.[9] A seroprevalence study performed in Ireland showed that IgG phase II seropositive women (11.2% of the 1209 women totally

screened) had more often a history of miscarriage or stillbirth.[19] An explanation for this discrepancy could be in differences in pathogenicity between different *C. burnetii* strains. Genotyping of Dutch samples is ongoing[20], but has not ended yet. Since in The Netherlands a relatively high number of chronic Q fever has been described in patients with aneurysms[21], it could be hypothesised that the strains involved in the Dutch outbreak are highly virulent for people with underlying vascular diseases, while pregnant women are relatively protected.

There are also studies in line with our results. Two large studies conducted in Q fever high-risk areas in The Netherlands and France did not find an association between seropositivity and complicated pregnancy outcome.[22,23] Furthermore, identifying nulliparity, maternal smoking during pregnancy and delivery of a boy as the strongest predictors for adverse obstetric outcome in a multivariable approach is similar to the results of previous studies.[24-26] The effect of BMI on obstetric outcome as described in the literature is, however, two-sided. On one hand, high BMI has been strongly associated with stillbirth.[24] On the other hand, low BMI has been associated with low birth weight and SGA.[26] For preterm delivery both low and high BMI have been shown to be risk factors.[27,28] Since preterm delivery and SGA are much more common than perinatal mortality, in our data low BMI turned out to be the risk factor with respect to the primary endpoint, which was a composite measure of preterm delivery, SGA and perinatal mortality. In our multivariate model vaginal blood loss in the second half of pregnancy had the strongest effect on the primary endpoint (RC 0.911, OR 2.47), however, this effect is more likely caused by clinical intervention than by its natural course.

The strength of this study is that it was performed on a large cohort of pregnant women with a high prevalence of *C. burnetii* seropositivity due to the enormous Q fever outbreak in The Netherlands. Furthermore, data collection was very complete. Therefore 1194 of the 1221 cases (98%) could be included in the final models. Generalisability to all pregnant women under supervision of primary health care in Q fever high-risk areas is expected since we included a relatively unselected group, predictors were clearly defined and easy to determine. Although large numbers of women were not willing to participate in the clustered RCT on *C. burnetii* screening, random refusal is expected since major patient characteristics, like maternal age and proportion of nulliparous women, are comparable with other large population based cohort studies from The Netherlands.[29,30] However, pregnant women with a non-Western ethnicity were underrepresented in our study population because only Dutch-

speaking women were eligible for inclusion. Therefore, the model should be interpreted with caution for this specific group.

In conclusion, prediction of adverse obstetric outcome in women who have been assessed as having a low risk for obstetric complications in the beginning of pregnancy is difficult using the well-known risk factors like nulliparity and smoking during pregnancy. *C. burnetii* seropositivity was not associated with obstetric complications in a multivariate setting and adding this variable to the prediction model did not improve the model's predictive capacity. Therefore, knowledge about *C. burnetii* antibody status is not useful in risk assessment.

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Chapter 7

Placental histopathology after *Coxiella burnetii* infection during pregnancy

Placenta 2011;33:128-131.

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ABSTRACT

Symptomatic and asymptomatic *Coxiella burnetii* infection during pregnancy have been associated with obstetric complications. We described placental histopathology and clinical outcome of five cases with asymptomatic *C. burnetii* infection during pregnancy and compared these cases with four symptomatic cases from the literature. In contrast with the symptomatic cases, we did not observe necrosis or active inflammation in the placentas of the asymptomatic women. Obstetrical outcome was more favourable in the asymptomatic cases than in the symptomatic cases. Asymptomatic and symptomatic *C. burnetii* infection during pregnancy are different entities with respect to placental histopathology and the risk of obstetric complications.

INTRODUCTION

Several European countries notified increasing numbers of human Q fever since 2007.[1,2] Q fever is a zoonosis caused by the intracellular bacterium *Coxiella burnetii*. It primarily infects ruminants and rodents, in which the infection is mainly associated with miscarriage and stillbirth.[3] Humans are predominantly infected by inhalation of contaminated aerosols.[4]

Up to 90% of pregnant women with antibodies suggesting recent infection with *C. burnetii* remain asymptomatic.[5] However, symptomatic and asymptomatic *C. burnetii* infection during pregnancy have been associated with obstetric complications, including miscarriage, preterm delivery and foetal death.[6,7] Placental infection assessed by polymerase chain reaction (PCR) or culture has been strongly related to these complications.[6] However, information about placental histopathology, in particular in asymptomatic cases, is lacking. Therefore, we described placental histopathology from women with asymptomatic *C. burnetii* infection during pregnancy. Subsequently, we compared our results with symptomatic cases described in the literature.

PATIENTS AND METHODS

Setting and participants

This study was embedded in a clustered randomised controlled trial about the effectiveness of a screening program for *C. burnetii* infection during pregnancy. In that study pregnant women living in Q fever high-risk areas in The Netherlands were serologically screened for *C. burnetii* infection. Details about the screening study are described elsewhere.[8] The study protocol was approved by the Medical Ethical Review Board of the University Medical Center Groningen (UMCG). All participants included in this study gave written informed consent to collect and analyse placental tissue and clinical outcome data.

Design

From women who participated in the intervention group of the screening trial and who had serological evidence for an acute infection, placentas were collected. An acute infection was defined as the presence (cut-off titre ≥ 1:32) of immunoglobulin (Ig)M accompanied with (rising) IgG during follow-up. Serology was performed with indirect immunofluorescence assay (IFA, Focus

Diagnostics, Cypress, CA, USA). Placentas were histopathologically analysed by one pathologist (AT) from the UMCG. Furthermore, *C. burnetii* specific real-time PCR was performed. Primers and probes used have been described earlier[9], other technical details are available on request.

Systematic review

A systematic review of the literature was done by searching PubMed and the references of the included papers following the PRISMA-guidelines. Our search was limited to human studies in English or Dutch. The search strategy was: ''Q fever OR *Coxiella burnetii*'' AND ''placenta''. First we pre-screened the titles and the abstracts; afterwards the eligibility of the studies was judged by reading the full-texts. Only studies describing human placental histopathology were included.

RESULTS

Seven of the 536 women in the intervention group of the screening trial had serological profiles suggesting an acute *C. burnetii* infection and were treated with antibiotics. Overall, five placentas were stored and send for re-evaluation to the UMCG, including two placentas from women with follow-up serology suggesting a previous infection. All cases were asymptomatic at the moment of screening. Clinical outcome and placental histopathology are summarised in the first part of Table 1.

The PubMed search resulted in 30 hits. Only 2 papers included data on human placental histopathology and were included. Two other reports were included based on the references. All included papers concerned case-reports of symptomatic acute or chronic Q fever cases.[10-13] Clinical outcome and placental histopathology of these cases are summarised in the second part of Table 1.

DISCUSSION

We showed that asymptomatic and symptomatic *C. burnetii* infection during pregnancy are different entities with respect to placental pathology and the risk of obstetric complications. Placental histology in the asymptomatic cases showed, in contrast with the symptomatic cases, no foci of necrosis or active inflammation. We only observed a few scattered fibrotic villi, which could be a

result of interruption of foetal blood flow or destruction of capillaries due to previous villitis.[14] The presence of low grade chronic villitis is a frequent finding in third trimester placentas and probably related to a maternal immune response directed against foetal antigens inherited from the father. Until present no microbiological pathogens have been linked to chronic villitis.[15] Whether placenta hypoplasia and pathology consistent with maternal vascular underperfusion are linked to *C. burnetii* infection is to our knowledge unknown.

In none of the placentas from asymptomatic cases *C. burnetii* could be detected with PCR. Previously this also has been shown for a larger cohort of 153 asymptomatic seropositive women[7], suggesting that the rate of placental infection during asymptomatic *C. burnetii* infection is very low.

Our findings are in line with animal studies. In cows, where Q fever is usually not clinically apparent, positive PCR on bulk tank milk is only rarely associated with histopathological inflammation of placentas.[16] On the other hand, in goats and sheep, in which *C. burnetii* infection is often associated with miscarriage and stillbirth, necrotising inflammation of placental tissue is a common finding.[17,18]

Various factors, including host immune response, cytokines and different strains of *C. burnetii*, have been suggested to play a role in the clinical manifestation and outcome of *C. burnetii* infection in both animals and humans, but further research is needed to find target points for prevention and treatment.[18-20]

In conclusion, after asymptomatic *C. burnetii* infection during pregnancy placental examination reveals no major pathology related to previous villitis, which is associated with a favourable clinical outcome. Symptomatic infection is a different entity. Obstetric complications in these cases can very well be explained by colonisation with *C. burnetii* and massive necrosis of the placenta.

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Table 1. Summary of patient characteristics and placental histopathology

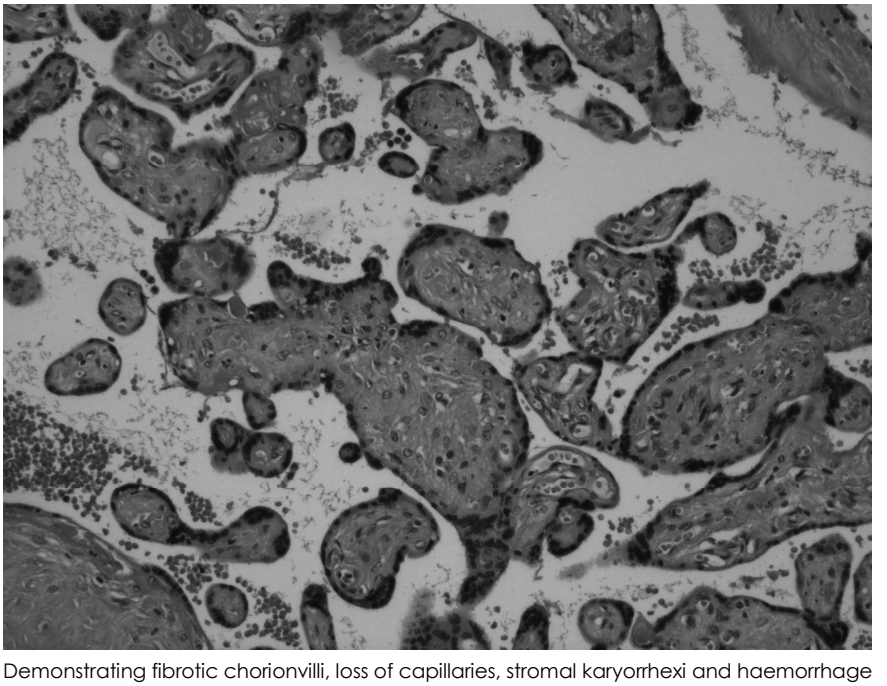
	Age (y)	Parity	Symptoms	Initial serological values[a]				Treatment
				IgM II	IgM I	IgG II	IgG I	
Asymptomatic patients								
1	31	0	None	1:1024	1:32	1:512	<1:32	Erythromycin
2	32	0	None	1:256	1:32	1:1024	1:128	Erythromycin
3	31	0	None	1:512	1:64	1:256	<1:32	Erythromycin
4	34	0	None	1:512	<1:32	1:128	<1:32	None
5	33	1	None	1:256	<1:32	1:128	<1:32	None
Symptomatic cases from the literature								
1 Reich-man et al. 1988	29	2	Fever, headache, weakness, sweating, purpuric rash	1:400	1:1600	1:1600	1:400	Tetracycline
2 Raoult et al. 1994	26	Un-known	Fever, cough	Unknown; seroconversion				Cotrimoxazole
3 Friedlan d et al. 1994	26	Un-known	Fever, fatigue, dyspnoea	Unknown; rising antibodies				Erythromycin post partum
4 Bental et al. 1995	28	Un-known	Fever, cough, arthralgia	1:1600	1:200	1:800	1:25000	Erythromycin/ rifampicin

Legend Table 1. y, years; Ig, immunoglobulin; wks, weeks; g, grams; PCR, polymerase chain reaction; NA, not applicable due to PCR inhibition; PPROM, preterm premature rupture of membranes.

Gestational age at delivery (wks+days)	Birth weight (g/ percentile)	Clinical outcome	*C. burnetii* present in placenta tissue?	Placental weight (g/ percentile)	Summery of placenta histology
42+0	4030/ 50-80th	Arrest of second stage of labour; uncomplicated caesarean section at term	PCR negative	Unknown	No significant pathology
37+0	2930/ 50-80th	Suspicion of solution placentae at term; emergency caesarean section	PCR negative	540/75-90th	No significant pathology
34+1	2170/ 50-80th	PPROM, retained placenta, postpartum haemorrhage	PCR negative	337/10-25th	Maternal vascular underperfusion; fibromuscular hyperplasia of stemvillus vessels; scattered fibrotic villi, low grade chronic villitis (Fig. 1)
39+6	3535/ 50-80th	Uncomplicated, at term	PCR negative	425/<10th	Placental hypoplasia
40+2	3535/ 20-50th	Congenital hydronephrosis, meconium stained amniotic fluid at term	NA	528/25-50th	Low grade chronic villitis
28	1000/ unknown	Induced labour because of maternal illness	Immunofluorescent stain positive	Unknown	Areas of necrosis
24	Unknown	Miscarriage	Immunofluorescent stain positive	Unknown	Multiple foci of necrosis
25	Unknown	Oligohydramnios, intrauterine foetal death	Immunocytochemical strain positive	Unknown	Severe necrotising villitis in 40% of the placental tissue
30	1300/ unknown	Premature labour, c. section because of transverse lie of the foetus	PCR positive	Unknown	No areas of necrosis or other gross pathology

[a] Serology of the asymptomatic cases was performed with indirect immunofluorescence assay (IFA, Focus Diagnostics, Cypress, CA, USA), measuring both IgM and IgG against phase I and II antigens. Serology of the symptomatic cases was performed with in-house assays.

Figure 1. Hematoxylin and eosin stain of placental tissue from asymptomatic case no 3.



Demonstrating fibrotic chorionvilli, loss of capillaries, stromal karyorrhexi and haemorrhage (magnification 10X).

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7

Chapter 8

Specificity of indirect immunofluorescence assay for the detection of *Coxiella burnetii* IgM during pregnancy

Submitted

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ABSTRACT

Since acute Q fever during pregnancy is an indication for long-term antibiotic treatment, accurate IgM phase II assays are indispensable. Seroprevalences in women from high (n=1229) and low-risk (n=180) Q fever areas were compared using indirect immunofluorescence assay (cut-off titre 1:32). The resulting IgM phase II seroprevalences of 4.3% and 0% (*P*=0.001), respectively, indicate 100% specificity.

INTRODUCTION

The incidence of Q fever, a zoonosis caused by the bacterium *Coxiella burnetii*, has enormously increased in several European countries since 2007.[1] Most of the infected individuals are either asymptomatic or present with a mild flu-like illness. However, *C. burnetii* may pose a serious threat to pregnant women because of the increased risk of chronic Q fever which can be complicated by endocarditis.[2,3] In addition, Q fever during pregnancy has been associated with obstetric complications.[3,4] Because of these complications, accurate diagnosis and knowledge about the significance of *C. burnetii* antibodies during pregnancy is indispensable.

Because of its simplicity and safety, the diagnosis of Q fever largely relies upon serology, of which indirect immunofluorescence assay (IFA) is the reference method.[5] One of the characteristics of *C. burnetii* is antigenetic phase variation; antibodies against two phases of antigens can be distinguished. After exposure to *C. burnetii* immunoglobulin (Ig)M against phase II antigen is the first antibody to appear, followed by IgM phase I, IgG phase II and IgG phase I.[5] For the diagnosis of acute Q fever demonstrating IgM phase II is therefore of great importance. Presence of IgG in the absence of or in combination with low titres of IgM suggests a previous infection. Test characteristics of IFA during pregnancy are not known. It is a well-known fact that serological assays for other infectious diseases during pregnancy, especially IgM assays, may produce false-positive results.[6,7] Since acute Q fever is an indication for long-term antibiotic treatment[3], knowledge about the specificity of IgM phase II assays is crucial to prevent unnecessary treatment during pregnancy.

A recent Dutch Q fever outbreak was hypothesised to be the result of highly infected dairy goat and sheep farms. This outbreak in The Netherlands was clearly defined in time and place.[8] This gave us the opportunity to compare sera drawn from pregnant women in high-risk areas with sera from pregnant women in low-risk areas. The objectives of this study were (1) to describe the seroprevalences of the different antibodies during pregnancy determined by IFA in a low and high-risk area, and (2) to estimate the specificity of IgM phase II IFA during pregnancy.

MATERIALS AND METHODS

Participants and setting

Women from high-risk areas

Sera from pregnant women living in Q fever high-risk areas were obtained from a clustered randomised trial (RCT) on screening for *C. burnetii* infection during pregnancy, which was conducted in The Netherlands between March 2010 and March 2011.[9] Fifty-five primary care midwife centres from regions with the highest Q fever incidences in 2009 and 2010 recruited pregnant women for participation. From women giving informed consent, a blood sample was taken between 20 and 32 weeks of gestation. The study protocol was approved by the Medical Ethical Review Board of the University Medical Center Groningen.

Women from low-risk areas

Sera from pregnant women who were not very likely exposed to *C. burnetii* recently, were obtained anonymously from the Centre for Infectious Diseases Friesland Izore, The Netherlands. Sera were taken around 12 week of gestation as part of a routine screening program for infectious diseases (hepatitis B virus (HBV), human immunodeficiency virus (HIV) and syphilis) from women living in the Dutch North-western province Friesland. In this province there were no dairy goat or sheep farms with *C. burnetii*-induced abortion waves (>5% abortions) and routine tests for the presence of *C. burnetii* in bulk tank milk, which were performed by the Dutch government between October 2009 and April 2010 as part of the preventive measures to curb the Q fever epidemic, were all negative.[8,10] To extra minimise the risk of true-positive samples, all selected sera were drawn in 2007, the year before there was a *C. burnetii*-associated abortion wave on a farm near the border of the province Friesland[10] and before the widespread Dutch Q fever outbreak (2008 and 2009).[8] Since none of the included women from the high-risk area were HIV, HBV or syphilis positive, only negative sera for those diseases were selected to improve comparability between the two groups. Furthermore, in line with the women from high-risk areas, we only included women under supervision of a primary care midwife. In The Netherlands, midwives working in primary health care are only allowed to supervise low-risk, singleton pregnancies in healthy women. By excluding sera from women under supervision of an obstetrician (secondary health care) we excluded women with auto-immune diseases or other underlying illnesses that are known to be associated with aspecific antibody formation.[7] Since the samples were obtained and screened

anonymously, specific consent of these participants for this study was not required.

Screening method

All sera were analysed with indirect immunofluorescence assay (IFA) in the laboratory of the Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands. IgM and IgG against phase I and phase II antigens (Nine Mile strain) were measured according to the manufacturer's instructions (Focus Diagnostics, Cypress, CA, USA). Each run included a positive and a negative control. In line with the cut-off values used in the clinical setting for the diagnosis of Q fever in symptomatic patients, titres ≥ 1:32 were considered positive.[11] Every positive sample was fully titrated. Samples with the titre 1:32 were re-analysed and judged by two laboratory workers to preclude aspecific reactions. Only if both agreed about positivity the sample was assessed as such.

Statistical analysis

Seroprevalences were calculated for the different antibodies by dividing the number of positive cases with the total number tested. Since no IgM phase II positive cases were expected in the low-risk areas, specificity of the assay was estimated using the formula: 100% – (IgM phase II seroprevalence of the low-risk group). Fisher's exact test was used to test the differences between the two groups. A two-sided *P* value of less than 0.05 indicated statistical significance. Statistical analyses were performed using PASW Statistics version 18.0 (SPSS inc. Chicago, Illinois, USA).

RESULTS

Within the clustered RCT (women from high-risk areas) 1229 women were routinely screened for the presence of *C. burnetii* antibodies. From the areas with a low risk of *C. burnetii* exposure 180 samples were randomly selected and tested. Seroprevalences are shown in Table 1. Specificity of the IgM phase II assay turned out to be 100% (95% confidence interval 98%-100%).

Table 1. Seroprevalences of the different antibodies per group

Antibodies present[a]	Women from high-risk areas (n=1229)	Women from low-risk areas (n=180)	*P*
Seropositive[b]	187 (15.2)	20 (11.1)	0.176
Any IgM	56 (4.6)	0 (0)	0.001
IgM phase I	10 (0.8)	0 (0)	0.626
IgM phase II	53 (4.3)	0 (0)	0.001
Any IgG	179 (14.6)	20 (11.1)	0.252
IgG phase I	34 (2.8)	4 (2.2)	0.810
IgG phase II	178 (14.5)	20 (11.1)	0.252
IgG phase II titre			
1:32	60 (4.9)	7 (3.9)	0.708
1:64	37 (3.0)	2 (1.1)	0.220
1:128	25 (2.0)	5 (2.8)	0.576
1:256	29 (2.4)	4 (2.2)	1.000
>1:256	27 (2.2)	2 (1.1)	0.571

Data are no. (%) of women. Ig, immunoglobulin.
[a] Cut-off titre 1:32 [b] Positivity for any IgM and/or IgG

DISCUSSION

As expected from a region without recent *C. burnetii* exposure we did not find IgM phase II positive cases in the low-risk area, in contrast to a 4.3% seroprevalence in the high-risk area (*P*=0.001). This finding suggests that *C. burnetii* IgM phase II IFA during pregnancy, using a cut-off of 1:32, is 100% specific. Still, the presence of IgM phase II should always be judged in relation to IgG, because both isotypes of antibodies will appear following an acute infection.[5] After combining the results for all four antibodies, only two positive IgM phase II results in the high-risk group (0.2%) were judged to be aspecific reactions by a medical microbiologist with much experience in this field (ACAPL), indicating a specificity slightly less than 100% with a positive predictive value over 96%.

The overall *C. burnetii* seroprevalence in both groups of pregnant women from a low-risk and a high-risk area was high, being 11.1% and 15.2% respectively, mainly caused by a high prevalence of IgG phase II. These seroprevalences seems to be higher than in non-pregnant Dutch populations.[12,13] Before the epidemic a seroprevalence of 2.4% was estimated for the general population[12] and during the outbreak in 2009 a seroprevalence of 12.2% was measured among blood donors.[13]

There are several reasons for the difference in seroprevalence between our pregnant women and the non-pregnant population. First of all *C. burnetii* exposure risk could be different, especially with respect to our low-risk group. We selected these women from an area without known infected dairy goat or sheep farms, but the province Friesland has many sheep farms. Previously in southwestern Germany, the seroprevalence showed a linear increase with sheep density.[14] Since IgG phase II can be positive over 10 years after the primary infection[15], possible undetected *C. burnetii* infected farms in the past could have led to an increased seroprevalence in human living in this area.

Another explanation could be the different characteristics of the hosts. During pregnancy sex hormones cause shifts of immunity from cell mediated to humoral, which could lead to higher immunoglobulin levels at baseline and in response to infection.[16,17] Whether the term of pregnancy, which differed between our low and high-risk group, is important in this setting is unknown. Furthermore, cut-off values in general and in specific during pregnancy are subjects of ongoing debate. Since we pursued a high sensitivity, we used a cut-off titre of 1:32 for all antibodies. The manufacturer noted that levels of phase II IgG <1:256 may be considered non-specific. If we would apply this to our test results the seroprevalences of phase II IgG in our high-risk and low-risk group would drop to 4.6% and 3.3%, respectively. Because *C. burnetii* is widespread in the environment and infected animals are mostly asymptomatic[8] we are unable to guarantee absence of previous exposure to *C. burnetii* in the low-risk group. Therefore specificity of the IgG assay using different cut-off values can not be given.

CONCLUSION

The findings of our study indicate a very high specificity of the IgM phase II IFA during pregnancy, using a cut-off titre of 1:32, both when solitary judged as well as in combination with other isotypes. The overall *C. burnetii*

seroprevalence in pregnant women from both the high and low-risk area was unexpectedly high, being 15.2% and 11.1% respectively.

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Chapter 9

Risks, trust and knowledge: determinants of pregnant women's decisions regarding participation in a future Q fever screening and treatment program during a large epidemic in The Netherlands

Prenatal Diagnosis 2011;31:814-820.

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ABSTRACT

Objective Contracting Q fever during pregnancy carries a risk of developing obstetric complications. The aim of this study was to gain insight into pregnant women's decisions regarding participation in a future Q fever screening and treatment program.

Methods Pregnant women (n=148) in Q fever high-risk areas in The Netherlands were recruited via midwives' practices and via an online panel for a cross-sectional questionnaire survey. The outcome measures included intention to participate in the program, Q fever exposure risk, perceived Q fever risk, trust in health professionals and authorities, disease-related knowledge and additional outcome measures.

Results Fifty-six percent of the respondents intended to participate in the screening and treatment program. The sole determinant of a higher intended program uptake was a more positive appraisal of program efficacy and convenience. This appraisal was in turn associated with perceived risk and knowledge.

Conclusion Women's appraisal of program efficacy and convenience, their disease-related knowledge and perceived Q fever risk seem to be crucial for their intended program uptake. A successful implementation of a Q fever screening and treatment program may thus depend on the benefits and downsides of the program, and on securing that women are aware of the risks of the disease.

INTRODUCTION

Q fever, a zoonosis caused by the bacterium *Coxiella burnetii*[1,2], has recently caused a large epidemic in a specific region in The Netherlands.[3] A total of 2357 human cases were notified in 2009.[3] Pregnant women have been described to be a risk group for Q fever.[4-6] Acute and chronic Q fever increases the risk of developing obstetric complications, such as foetal growth restriction, low birth weight, premature delivery, oligohydramnios, stillbirth and spontaneous abortion.[4-6] Veterinary measures have been implemented to control the Q fever epidemic in The Netherlands. However, it has been suggested that these veterinary measures may not decrease Q fever incidence levels.[3] Public health preventive measures may therefore be essential to prevent potential adverse obstetric outcomes. Accordingly, a large study has been undertaken to assess the costs and effectiveness of implementing a specific Q fever screening program for pregnant women in The Netherlands.[7] Such program in which pregnant women are offered maternal blood serum screening for *C. burnetii* has been advised under epidemic conditions, as Q fever during pregnancy is commonly asymptomatic.[8] Administering appropriate cotrimoxazole treatment to women who test positive for Q fever would then prevent Q fever-induced obstetric complications.[5]

The effectiveness of any screening and treatment program for Q fever depends on the pregnant women's uptake and compliance. Although some studies have examined the influence of various determinants on the uptake of prenatal screening aimed at detecting genetic diseases[9], few studies have looked at the decisions of pregnant women regarding serum screening for infectious diseases. To our knowledge, no studies have been conducted on pregnant women's decisions regarding screening for emerging infectious diseases under epidemic conditions. The present study aimed to gain insight into the decisions of pregnant women regarding participation in a future screening and treatment program for Q fever. The study focused on Q fever exposure risk, perceived Q fever risk, knowledge about the disease, and trust in health professionals and authorities. Given the relatively new, emerging nature of this infectious disease, we expect that risks and knowledge are particularly relevant determinants of pregnant women's decisions regarding participation in the program. Q fever exposure risk had our attention, as this real risk might have an influence on perceived risk. As Sjoberg stated, 'several factors have been proposed for the explanation of perceived risk'.[10] A primary candidate is, of course, real risk. Subsequently, perceived risk may have an influence on

pregnant women's decisions regarding participation in the program, as it is a central determinant of intentions in many health-behaviour-predicting theories.[11] Moreover, perceived risk has been found to be associated, although weakly, with pregnant women's attitude towards undergoing prenatal screening for Down syndrome.[12] Knowledge of Q fever may also be relevant, as pregnant women might be unfamiliar with this disease. Knowledge about the condition being screened for has been described to influence the uptake of prenatal screening for Down syndrome.[13] Moreover, trust in health professionals and authorities was considered to be relevant, given their vital role in implementing central screening programs. The level of trust in public health authorities and health care professionals has, for instance, been described to positively influence parental protective behaviour in studies on parental uptake of traditional MMR vaccination.[14,15] Furthermore, the current study looked at the influence of some other determinants obtained from general health behaviour predicting theories (e.g. protection motivation theory[16]). This study identified the key determinants of a successful implementation of a maternal serum screening and treatment program for Q fever by public authorities.

METHODS

Study sample and procedure

The study comprised a cross-sectional questionnaire survey among pregnant women who lived in Q fever high-risk areas in The Netherlands (Noord-Brabant, Gelderland and Limburg). Women were recruited in two ways, either via their midwifes' practice or via an existing online panel. Ten midwives' practices were selected in municipalities where Q fever incidence in 2009 was over 5 per 100,000 inhabitants.[17] These midwives' practices were asked for their cooperation by the National Institute for Public Health and the Environment. Eight practices recruited respondents by handing out invitation leaflets to pregnant women who came to the practice for a routine visit. The invitation leaflet gave details about the study and included a web address where a link to the web-based questionnaire could be found (www.examine.nl). Two other midwives' practices directly distributed paper-form questionnaires during consulting hours. Subsequently, the respondents recruited via the midwives' practices consisted of a web-based group and a paper-based group. Separately, a group of pregnant women received a questionnaire via an existing online research panel [Flycatcher (http://www.flycatcher.eu/)]. This

research panel is representative of the general Dutch population. For this study, a sample of panel respondents was selected based on postal codes to match the areas where respondents were recruited via midwives' practices.
The resulting three groups of pregnant women will be denoted as 'web-based,' 'paper-form' and 'panel' groups. A screening strategy study for Q fever, simultaneously with the present study, which was conducted between April and May 2010, during pregnancy, was held in the same region.[7] It was secured that no respondents of that study also participated in the current study.

Materials

The questionnaire used in the study contained the sections with questions pertaining to background characteristics and outcome measures as well as a section dedicated to providing information about the Q fever screening and treatment program that was offered. As this program is not yet implemented, the offer in this study was hypothetical. The information that respondents received about the program was formulated in correspondence with the authors of the ongoing screening strategy study for Q fever.[7] This information included the screening and treatment procedure, and the duration, benefits and potential side effects of antibiotic treatment. The questionnaire addressed the following outcome measures (in order of appearance in the questionnaire).

Knowledge about Q fever and Q fever during pregnancy was tested by six questions (answer categories 'true,' 'false' and 'don't know').

Affected acquaintance Respondents were asked if they knew someone who had contracted Q fever (one item).

General perceived risk of conditions during pregnancy which are generally known to affect baby's health (i.e. alcohol, smoking, toxoplasmosis) was measured by six items. Addition of the responses on these items resulted in a combined scale with Cronbach's α=0.862. Cronbach's α is a measure that reflects the reliability of outcome measures that are constructed by adding the scores of the answers of multiple items. It is generally agreed that a Cronbach's α of approximately 0.7 or higher indicates a satisfactory internal consistency on a group level.[18]

Perceived Risk of Q fever during pregnancy was measured by four items (Cronbach's α=0.632) which assessed the perceived foetal vulnerability, perceived severity of Q fever-induced obstetric complications, perceived own vulnerability of contracting Q fever during pregnancy, and perceived own

vulnerability compared to other pregnant women of the same age (perceived relative risk).

General anxiety was assessed by two items (Cronbach's α=0.785) regarding anxiety of stillbirth or of physical defects of the baby, derived from a pregnancy related anxiety questionnaire.[19]

Anxiety was measured by two questions (Cronbach's α=0.985) about the respondent's worries about Q fever-induced obstetric complications and the influence of Q fever on their baby's health.

Preventive measures Respondents were asked if they had already adopted measures to prevent Q fever during pregnancy (one item, e.g. avoidance of contact with goats and sheep during pregnancy).

Decisions regarding participation Respondents were asked if they intended to participate in the screening and treatment program, if this program would be organised and recommended by public authorities at present (one item) or would be organised and recommended in future (one item). In one open-ended question, respondents were then asked for the main reasons of their decision regarding participation in the program. Furthermore, respondents were asked how certain they were of this decision (one item).

Appraised efficacy and convenience Respondents were asked by six items (Cronbach's α=0.715) how they appraised the efficacy of the Q fever screening test (e.g. regarding prevention of Q fever-induced obstetric complications), of the antibiotics treatment and of the screening and treatment program in total, and whether they thought the treatment would be inconvenient. Appraised efficacy and inconvenience have been described to be predictors for health protective behaviour in terms of response efficacy and response costs in the protection motivation theory.[16]

Trust in the communication and competence of first line health care professionals and public authorities regarding Q fever during pregnancy was assessed by ten items (Cronbach's α=0.908), in analogy with trust items described for environmental risks.[20]

Exposure risk was estimated based on six items which assessed known risk factors for contracting Q fever: the proximity of a Q fever-affected dairy goat farm,[21] occupational risk[22-24] and direct contact with Q fever-infected ruminants[25] or with materials originating from these animals.[26] A risk profile was calculated based on the number of risk factors (no vs one or more).

The information about the screening and treatment program was presented after the question about other preventive measures. The questionnaire was

pilot-tested among pregnant women in Q fever high-risk areas and subsequently slightly revised to enhance comprehensibility.

Analysis

Descriptive analyses were performed for each variable. As the distributions of the variables anxiety and exposure risk were skewed to the right, these variables were dichotomised. The main reasons of participants' intentions for participation in the screening and treatment program or for no participation were classified and categorised by the first and third author separately. The determinants of intention to participate at present were identified by simple and multiple logistic regression analyses (backward and forward selection). To establish the determinants of appraised efficacy and convenience, general linear model multiple regression analysis (backward selection) was used. Demographic, obstetric, general anxiety and general perceived risk variables were included one-by-one in these logistic and general linear model analyses. The association between exposure risk and perceived risk items was examined by independent sample *t* -tests and linear-by-linear chi-square tests. For all outcomes (responses or found associations) differences between the three groups of respondents (web-based, paper-form or panel data) were checked by analysis of variance (ANOVA) and chi-square analysis. All significance tests performed in this study were two-sided (α=0.05) and analyses were conducted using SPSS version 17.0 and 18.0.

RESULTS

Sample descriptives

In total, 148 respondents completed the questionnaire, of which 61 were web-based (response rate 9%), 36 paper-form (response rate 36%), and 51 panel respondents (response rate 73%). The majority of the outcome measures did not differ between the web-based, paper-form and panel group (ANOVAs and chi-square analyses, $P>0.05$). These three groups solely differed in knowledge and gestational age. The paper-form group had a lower knowledge score (ANOVA, $F=8.89$, $P<0.001$) compared to the other groups, and had eight weeks' higher gestational age than had the panel group (ANOVA, $F=7.79$, $P=0.001$). The data source had no influence on the significance of the relationship between knowledge and intention to participate, or that of the association between knowledge and appraised efficacy and convenience. Table 1 presents demographic and obstetric

characteristics of the total sample. The majority of the respondents (94%) had heard about Q fever. Although most respondents (71%) were aware that pregnant women are a risk group for Q fever, a minority (35%) had heard about the effects of Q fever on the pregnancy outcome. Only 4% was aware that Q fever during pregnancy could be asymptomatic, whilst 35% incorrectly assumed that Q fever is invariably symptomatic and the other respondents indicated that they did not know the correct answer. The descriptive results of exposure risk, perceived Q fever risk, knowledge, trust, and additional determinants are shown in Table 2. The mean score of general anxiety was 3.3 (range 0–10, SD=0.15). General perceived risk had a mean score of 26.0 (range 0–32, SD=0.41).

Table 1. Demographic and obstetric data of the respondents

Demographic and obstetric variables	Total sample[a]
Age (years)	31.3 (4.5)
Gestational age (weeks)	25.1 (10.1)
Educational level, n (%)	
Low	9 (6.6)
Middle	50 (36.8)
High	77 (56.6)
Religion, n (%)	
None	56 (40.9)
(Roman) Catholic	64 (46.7)
Protestant	8 (5.8)
Other	9 (6.6)
Parity, n (%)	
Primiparous	69 (50.4)
Multiparous	68 (49.6)

Data are mean (standard deviation) unless otherwise specified.

[a] Because of missing values, the total number of respondents (n) differs for each variable.

Table 2. The descriptive results for perceived risk, trust, knowledge, exposure risk, and other determinants

Determinants	Total sample[a]	Range[b]
Perceived risk	12.2 (0.24)	0–21
Trust	39.0 (0.50)	10–50
Knowledge	14.8 (1.7)	6–18
Exposure risk, n (%)		
No risk factors	93 (68)	
One or more risk factors	44 (32)	
Appraised efficacy and convenience	11.8 (0.21)	0–20
Affected acquaintance, n (%)		
No	117 (84)	
Yes	23 (16)	
Preventive measures, n (%)		
No	124 (87)	
Yes	18 (13)	
Anxiety, n (%)		
None/little	113 (80)	
Considerable/much	28 (20)	

Data are mean (standard deviation) unless otherwise specified.

[a] Because of missing values, the total number of respondents (n) differs for each determinant.

[b] Ranges are presented for continuous determinants.

Pregnant women's decisions regarding participation

More than half of the respondents intended to participate in a Q fever screening and treatment program at present (Table 3). The answers about the intention to participate in the future and the intention to participate at present were strongly associated Pearson's $\chi 2=77.1$, $P<0.001$). Respondents were quite certain about their decision to participate in the program (Table 3). The most

frequently mentioned reasons of having no intention to participate (answer categories 'absolutely not' and 'probably not') were 'exposure risk perceived to be low' (22%); 'side effects of antibiotics not known or not yet known' (19%); 'own and baby's status perceived as healthy' (15%) and 'anxiety of the consequences of participation' (7%). The major reasons of the intention to participate (answer categories 'probably' and absolutely') were 'baby's health' (36%), 'certainty of not having Q fever' (13%), 'excluding health risk' (10%) and 'own health' (10%).

Table 3. Decisions of respondents regarding their participation in a Q fever screening and treatment program

Decisions regarding participation	Total sample[a]
Intended to participate in future, n (%)	
Absolutely not	7 (5.1)
Probably not	23 (16.7)
May be	29 (21.0)
Probably	57 (41.3)
Absolutely	22 (15.9)
Intended to participate at present, n (%)	
No	62 (44)
Yes	79 (56)
Certainty of decisions regarding participation	4.2 (1.4)[b]

Data are mean (standard deviation) unless otherwise specified.

[a] Because of missing values, the total number of respondents (n) differs for each variable.

[b] Range 0–6.

Determinants of intention to participate

Respondents' trust in health professionals and authorities regarding Q fever during pregnancy had no influence on their intention to participate at present (Table 4). Exposure to Q fever risk factors and perceived risk of Q fever during pregnancy were also not related to the intention to participate (Table 4). Appraised efficacy and convenience was the sole determinant of the intention to participate (Table 4). Respondents' intention to participate was

higher when they appraised the screening and treatment program as more efficacious and less inconvenient (Table 4). Demographic, obstetric, general anxiety and general perceived risk variables had no influence on the relationship between intention and appraised efficacy and convenience.

Table 4. Multiple logistic regression analysis results of the associations between the determinants and the intention to participate in the Q fever screening and treatment program at present

Determinants	β-value	SE	*P*
Perceived risk	0.001	0.10	0.99
Trust	0.06	0.04	0.14
Exposure risk	0.12	0.54	0.37
Knowledge	− 0.13	0.15	0.87
Appraised efficacy and convenience	0.86	0.16	<0.001
Affected acquaintance	0.95	0.73	0.19
Preventive measures	1.18	0.86	0.17
Anxiety	− 0.37	0.76	0.63

SE, standard error.

Determinants of appraised efficacy and convenience

The independent determinants of appraised efficacy and convenience were perceived risk and knowledge. Respondents, who perceived the risk of Q fever as high, appraised the program as more efficacious and less inconvenient (general linear model, β=0.229, SE=0.074, *P*=0.002). A higher knowledge about Q fever in general and Q fever during pregnancy was associated with a higher appraised efficacy and convenience (general linear model, β=0.284, SE=0.119, *P*=0.018). Demographic, obstetric, general anxiety and general perceived risk variables had no influence on the significance of these associations.

Exposure risk and perceived risk

Linear-by-linear chi-square tests revealed that respondents who had a higher exposure risk perceived the relative risk of contracting Q fever during pregnancy compared to other pregnant women of the same age as higher (χ2=6.72, *P*=0.012). However, an increased exposure to risk factors had no

influence on the perceived risk of contracting Q fever during pregnancy (independent samples *t* -test: $t=-0.741$, SE=0.527, $P=0.460$).

DISCUSSION

More than half of the pregnant women in Q fever high-risk areas included in the present study intended to participate in a Q fever screening and treatment program, if this program would have been implemented at present. The most important direct determinant of this intention was found in the pregnant women's appraisal of efficacy and of practical convenience of the screening and treatment program. This appraisal, in turn, differed among women depending on the perceived risk of Q fever and on the level of knowledge about Q fever. Trust in health professionals and authorities and actual exposure to Q fever risk factors were not associated with pregnant women's intention to participate in the program.

A positive appraisal of efficacy and convenience of the maternal Q fever screening and treatment program seems to be crucial for a women's intention to participate. This is in line with the finding that response efficacy influences adherence to medical treatment regimens in general.[27] In our study, about one quarter of the women who did not intend to participate had doubts about the side effects of the antibiotic treatment or was afraid of the consequences of participation. The importance of such appraised efficacy and convenience would dictate that information materials about Q fever screening should be very clear about the likelihood of the expected benefits and possible downsides of screening and treatment.

While the women's level of knowledge about Q fever had no independent influence on their decision regarding participation in the screening and treatment program, it had an indirect impact on this decision by influencing the appraisal of the program efficacy and convenience. Pregnant women who knew more about Q fever appraised the efficacy and convenience of the screening and treatment program more positively. This finding may reflect that awareness about Q fever and the potential of obstetric complications is generally limited. This is particularly illustrated by the commonly found incorrect assumption that Q fever during pregnancy would be invariably symptomatic. Such misconception would likely reduce the perceived need for serum screening unless a women experiences symptoms.

The intention of pregnant women to participate in the screening and treatment program was not directly influenced by perceived risk, which is in

contrast with the positive effect of perceived risk on intentions and behaviours in numerous studies based on the protection motivation theory.[27] However, women who perceived the risks of Q fever as higher had a more positive appraisal of the efficacy and convenience of the screening and treatment program. The finding that perceived Q fever risk and knowledge about Q fever in general and Q fever during pregnancy influenced the appraisal of these program characteristics, may suggest that for a new and somewhat unfamiliar disease pregnant women's appraisal of the efficacy and convenience of screening and treatment may particularly be influenced by either having some basic knowledge about the disease or by perceiving it as relatively risky.

The influences of other determinants on the intention to participate failed to reach statistical significance. Pregnant women's trust in the communication and competence of health professionals and authorities did not affect the intention to participate in the program. This finding is in contrast with previous studies on trust and parental protective behaviour, for example in studies on MMR immunisation.[14,15,28,29] In the present study, the pregnant women overall expressed a considerable level of trust about the topic in health professionals and authorities. Trust may therefore either play only a minor role in these decisions, or the trust may vary more when screening and treatment involve more well-known diseases.

Pregnant women's intention to participate in the screening and treatment program was also not influenced by Q fever exposure risk (based on, for instance, the proximity to an affected farm). This finding is in accordance with research on HIV maternal screening, as pregnant women exposed to few HIV risk factors did not differ in acceptance of screening with pregnant women exposed to multiple risk factors.[30] Moreover, it has been argued that, compared to actual risk, perceived risk has more influence on the uptake of prenatal tests.[9]

At this, our findings suggest that the relation between exposure risk and perceived risk is primarily a relative one. While women who were exposed to multiple risk factors perceived their risk of contracting Q fever as relatively higher compared to other pregnant women of the same age, their general risk perception about Q fever was similar. Although pregnant women thus seem well aware of their increased exposure risk, they may still perceive their total risk of subsequent obstetric complications in absolute terms as low.

This study has some potential limitations. First, while the regional Q fever epidemic provided a unique opportunity for our study, the locally confined nature of this epidemic meant that a relatively small number of the respondents were available for recruitment in the study. In addition, the

response rates for the groups that were recruited via the midwives' practices were fairly low compared with some other studies about attitudes towards prenatal screening.[12,31] An important reason for this may lie in the passive recruitment approach of our study, which consisted merely of distributing information leaflets and involved no active recruitment or discussion about Q fever or the screening and treatment program during the actual consultation with the midwife. While the low response rate may limit the generalisability of our findings, the results show that the outcome measures were similar across the different response groups, suggesting that the web-based and paper-form group were comparable to the panel group which had a relatively high response rate. Moreover, the demographic and obstetric characteristics of the total sample are comparable to that of previous large and representative studies among pregnant women in The Netherlands[12,32], although slightly more higher educated respondents were included in the study. This does not suggest that participation in this study was restricted to a specific subgroup of women. A second limitation may come from the fact that the respondents had to make a decision about participation in a hypothetical screening and treatment program. While this hypothetical nature could affect decision-making, the high certainty that women expressed about their choice suggests that few women felt in doubt about their current preference.

CONCLUSION

This study demonstrates that the decision to participate in a new screening and treatment program for Q fever is firstly determined by the pregnant women's appraisal of efficacy and convenience of the program. In turn, this appraisal is highly influenced by the prior knowledge that women have of the disease and by the risk they perceive it to carry for them and their child. Decisions were not influenced by actual exposure risk or by trust in health professionals and authorities. The success of implementing a screening and treatment program for Q fever may thus hinge on first the practical downsides and benefits of the program and second on securing that women are aware of the risks of the disease.

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Chapter 10

Effectiveness of the Q fever vaccine: a meta-analysis

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ABSTRACT

In The Netherlands, the number of notified human Q fever cases showed a steep increase over the last three years and is not expected to disappear in the next few years. Since vaccination might be an option to prevent Q fever cases in the general population, evidence is needed about its effectiveness. We therefore conducted a meta-analysis to determine the evidence base for effectiveness for Q fever vaccination in human populations. We calculated Mantel-Haenszel risk ratios and we used the following formula to calculate the vaccines effectiveness: (1 − mhRR) × 100%. Although individual and the pooled estimates showed a high effectiveness of Q fever vaccine, conclusions for the general population cannot be confidently drawn about vaccine effectiveness due to potential flaws in the design of the studies and the selected group of study participants.

INTRODUCTION

In The Netherlands, the number of notified human Q fever cases, caused by *Coxiella burnetii*, showed a steep increase over the last three years, with 168 versus 2357 new cases in 2007 and 2009, respectively.[1] Despite many measures being taken to prevent further transmission in The Netherlands, it can be expected that Q fever cases will occur in the next few years.[1] This is a serious hazard not only for those at high occupational risk to get the disease, but also to other vulnerable groups, such as pregnant women, immunocompromised persons and those with pre-existing cardiac valve- or vessel-defects.[2]

Currently only one Q fever vaccine (Q-Vax, Commonwealth Serum Laboratories Limited) is available for humans. This vaccine is registered in Australia and is there used in the population that has the highest occupational risk (mainly abattoir workers). Since vaccination with Q fever vaccine might be an option to prevent symptomatic and asymptomatic cases of Q fever in the general population, evidence is needed about its effectiveness. In 2007, a paper discussing the effectiveness of human Q fever vaccine was published.[3] However, although this study gave a good overview of literature, it did not aim to conduct a systematic analysis of current evidence for Q fever vaccine effectiveness.

We therefore conducted a meta-analysis to determine the evidence for the effectiveness of Q fever vaccination in humans in a systematic way. Furthermore, as studies on the effectiveness of Q fever vaccination were often small and probably biased, we aimed to assess bias by using the assessment criteria for randomised controlled trials and observational studies.

METHODS

A review of literature was done by searching PubMed and the references of included papers. Our search was limited to human studies in the English language. The search strategy was: ((Q fever OR *Coxiella burnetii* OR *C. burnetii*) AND (vaccination OR vaccine OR immunised OR immunisation)). First we pre-screened the titles and the abstracts; afterwards the eligibility of the studies was judged by reading the full-text. Only the studies that used Q fever vaccine in human and gave information about the clinical outcome and reported the raw data were included in the analysis. The final analysis was performed on the effectiveness of Q-Vax (CSL Limited) vaccine.

The design and possible limitations of the studies were assessed using criteria for randomised controlled trials[4] and longitudinal non-randomised observational studies.[5] As the main possible limitations we considered bias because of information, selection or confounding, which may lead to the over- or underestimation of the vaccine effectiveness.

The Mantel-Haenszel risk ratio (mhRR) was calculated after pooling the raw data by using Episheet by Rothman.[6,7] Vaccine effectiveness was calculated by the following formula: (1 − mhRR) × 100%.

RESULTS

Results of the search

The first search resulted in more than a hundred hits. Only five articles met our inclusion criteria, and three extra papers were included after screening the references (Fig. 1). We had to exclude one paper[8] that described an interim analysis as we included the complete study in our meta-analysis.[9] Finally, our search resulted in seven studies containing the raw data about the effectiveness of the Q fever vaccine.[9-15] Four of them contained the raw data about the effectiveness of Q-Vax (CSL Limited).[9,10,13,15]

We included three retrospective cohort studies[10,13,14], one prospective cohort study[9], one randomised controlled trial[15] and two experimental studies.[11,12] Except for the volunteers in the experimental studies, the study population consisted of persons who are at risk to get Q fever due to their profession, mostly abattoir workers and laboratory staff.

The summary of the included studies can be found in Table 1.

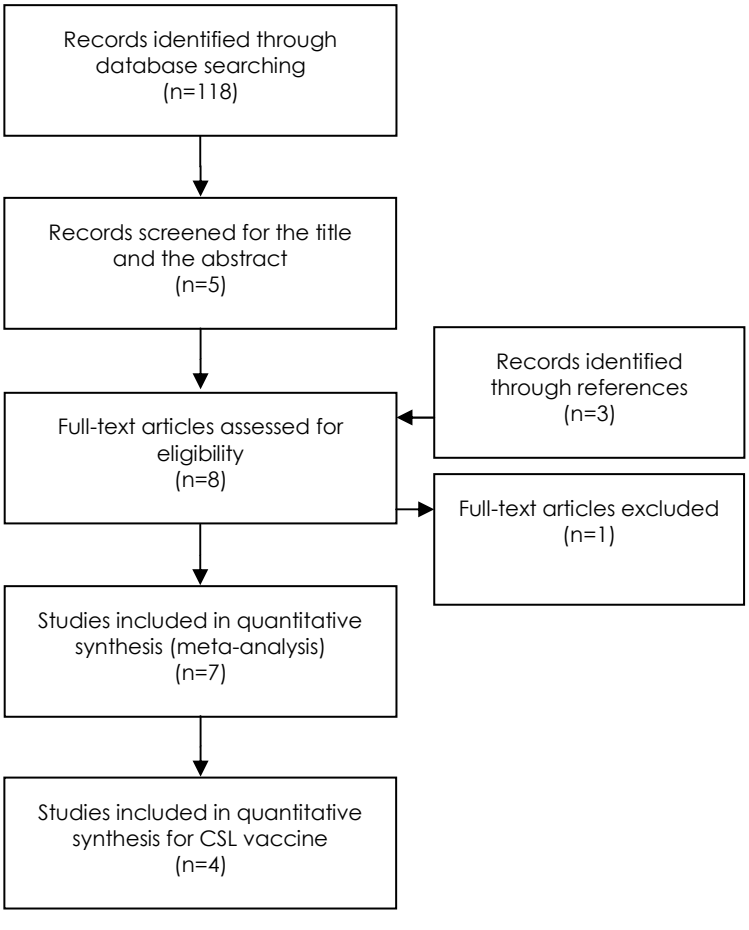
Assessment of vaccine effectiveness

All of the studies showed a protective effect of the vaccine against Q fever (ranged between 91 and 100%). The overall effectiveness of the vaccine as calculated after pooling the raw data was 97% (95% confidence interval 94–99%).

The incubation time of Q fever is around 15 days. Therefore, those who developed clinical signs and symptoms of Q fever within 15 days after vaccination could be considered to be vaccinated within the incubation time of a natural infection. After excluding those cases, the vaccine effectiveness increased to 99% (95% confidence interval 96–99.7%).

The effectiveness of Q-Vax (CSL Limited) vaccine was 98% (95% confidence interval 94–99%), and reached 100% after excluding the cases that occurred within 15 days after vaccination.

Figure 1. Flow diagram



Assessment of bias

One of the problems in the reviewed studies was possible bias due to the inclusion and exclusion criteria of vaccinees and nonvaccinees. In six of the reviewed studies the subjects were excluded from receiving Q fever vaccination when they had a positive antibody titre (CF titre ≥ 2.5) and/or positive skin test[9,10,12,13,14,15]; however there were exceptions and in some cases

the thresholds of serological and/or skin tests were not given.[10,11,12,14] In three studies the inclusion and exclusion criteria for nonvaccinees were not given or it was different from the criteria used for vaccines.[10,11,13] The inclusion of skin- and/or seropositive nonvaccinees might have led to underestimation of vaccine effectiveness as persons with positive markers are thought not to be at risk for Q fever infection.

Furthermore, vague or even absent case definition might have led to both under- and overestimation of vaccine effectiveness due to lack of objective assessment. Only in one of the reviewed studies Q fever case definition was properly described and included both a list of clinical symptoms and the cut-off values for serological markers.[13] Three studies also used serological markers to confirm suspected Q fever cases[10,11,15]; however the detailed description, including the list of symptoms and the cut-off points of serological markers was missing. A couple of studies did not provide any case definition. Only one of the reviewed studies was a blinded study.[13]

The absence of the description of the baseline characteristics of both vaccinees and nonvaccinees might have led to bias as well. The description of baseline characteristics, such as gender or age, of vaccinees and nonvaccinees was poor or absent in six studies.[10-15] For example, according to the National Q fever management program in Australia, the incidence and vaccination against Q fever is higher in males than in females.[16] There is already some evidence from animal studies that females are less susceptible to Q fever infection than males due to female hormones.[17] Such differences in the distribution of gender between vaccinees and nonvaccinees at baseline therefore might lead to bias. Only one of the reviewed studies provided a sufficient description of baseline characteristics.[9]

DISCUSSION

Individual studies showed that the effectiveness of the vaccine against Q fever is very high, without exceptions.[9-15] The same high vaccine effectiveness was found after pooling the raw data. Even when cases that occurred within 15 days after vaccination were included, the vaccine effectiveness was very high. However, the designs of the included studies had some potential flaws.

Different inclusion and exclusion criteria for vaccines and nonvaccinees, inclusion of seropositive nonvaccinees, vague or absent Q fever case definition, and differences in baseline characteristics of vaccinees

and nonvaccinees might have led to biased results of Q fever vaccine effectiveness.

Another major problem was the selected study sample: there were two studies performed on volunteers, four of the studies focused on abattoir workers and one study focused on laboratory staff. Although information about the demographic characteristics was limited, the study sample was relatively young. At least in three of the reviewed studies the mean age was around 30 years.[9,10,13]. Furthermore, the authors of the reviewed studies did not give information about the health status of the study participants. Still, as the study subjects were abattoir workers, laboratory staff and volunteers, it seems likely that they were relatively healthy. This creates problems to generalise the results in different populations. Additionally, it is unclear for how long the vaccine is protective against Q fever, and whether this protection is the result of vaccination in combination with a constant exposure to *Coxiella burnetii*. It was shown that the number of Q fever cases decreased with longer employment at the abattoir.[10]

CONCLUSION

In all, the vaccine effectiveness in groups with a high risk for Q fever seems to be very high. However, due to the selected study population and the absence of a proper description of the studied samples and study procedures, it is not possible to generalise our results and draw conclusion about the effectiveness of Q fever vaccine in the general population or in specific groups of patients. One of the important goals for the future should be decreasing Q fever incidence and prevention of related complications in persons who are not at constant exposure, but might be more vulnerable, such as pregnant women, immunocompromised persons or those with pre-existing cardiac valve- or vessel-defects.

It seems likely that the vaccine against Q fever might decrease the incidence of Q fever in these specific groups and in the general population as well. However more blinded, randomised and unbiased research about its effectiveness is needed.

Table 1. Description of studies included into meta-analysis.

	Ackland et al.[10]	Benenson[11]	Gilroy et al.[13]
Used Q-fever vaccine and dosage	Q-Vax, CSL (3 batches of 30 µg and 1 batch of 20 µg)	Formalin-killed Ether-extracted Henzerling strain Q-fever vaccine (3 x 1 ml)	Q-Vax, CSL
Study design	Retrospective cohort study	Experimental study	Retrospective cohort study
Intervention for control group	-	-	-
Setting, study population	3 Australian abattoirs, workers	USA, men volunteers	1 Australian abattoir, workers
Exclusion and inclusion criteria for vaccinees	Exclusion: positive serology (CF titre >=2.5) or skin test positive (presence of induration at 5-7 days); with a few exceptions	None	Inclusion: negative serology (CF titer <2.5) and skin test negative (7 days after the test)
Exclusion and inclusion criteria for nonvaccinees	Not given, but most likely both, who have positive and negative markers for Q-fever	None	None
Case definition	"the pattern of symptoms and signs conformed to the description of clinical Q fever" and "serological evidence indicating current or quite recent infection with *C. burnetii*"	"developing clinical disease"; "showing complement-fixing antibodies"	Confirmed case: ≥4 increase in antibody titre to phase II antigen (AG) by CFT or a positive IgM titer (≥80) to phase II AG by IFT. Suspected case: At least 4 of the following symptoms: fever, sweats, rigorous, fatigue, headache, myalgia, arthralgia, cough; serological tests negative or not available.
Number of cases among vaccinees	2[c]/2553	2/27	0/19
Number of cases among nonvaccinees	55/1365	8/10	7/68
Effectiveness (RR, CI 95%)	98% (92%-99%)	91% (64%-98%)	100%
Effectiveness[b]	100%	-	-
Limitations	1. Vague definition of cases 2. Exceptions in inclusion/exclusion of cases 3. No sufficient description of the baseline characteristics of vaccinees and nonvaccinees	1. Vague definition of cases 2. No sufficient description of the baseline characteristics of vaccinees and nonvaccinees 3. No randomisation or allocation procedures described 4. No pre-vaccination screening	1. No description of baseline characteristics of vaccinees and nonvaccinees

CFT, Complement Fixation Test; IFT, Immunofluorescence Test.

[a] These studies are described in review papers by other authors.[12,14]

[b] After excluding those who got ill within 15 days after receiving Q fever vaccine.

[c] Q fever cases occurred within 15 days after vaccination.

Marmion et al.[9]	[a]Philip[14]	[a]Richard B. Hornick[12,14]	Shapiro et al.[15]
Q-Vax, CSL (1X 30µg)	Q58-A (1X 22µg 1ml)	Q-Vax, CSL (1X 30µg)	Q-Vax, CSL (1X 30µg)
Prospective cohort study	Retrospective cohort study	Experimental study	RCT, double blind, crossover
-	-	-	Flu-vax 0.5 ml
1 Australian abattoir, workers	Laboratory staff	USA, volunteers	3 Australian abattoirs, workers
Inclusion: negative serology (CF negative at <2.5) and skin test negative	Inclusion: skin test negative	Inclusion: negative serology	Volunteers; Exclusion: positive serology and skin test positive
Both; but possibility to see the raw data with the same inclusion criteria as for cases	Inclusion: skin test negative	Inclusion: negative serology	Volunteers; Exclusion: positive serology and skin test positive
Not given	Not given	Not given	Suspected Q fever cases tested by CFT, IFT
2[c]/690	0/282	2/83	0/98
7/61	2/37	5/6	7/102
97% (88%-99%)	100%	97% (88%-99%)	100%
100%	-	-	-
1. No case definition 2. The allocation procedure between vaccinees and nonvaccinees not described	1. Insufficient case definition 2. No information about the baseline characteristics of vaccinees and nonvaccinees 3. No thresholds for skin tests	1. Insufficient case definition 2. No information about the baseline characteristics of vaccinees and nonvaccinees 3. Inclusion criteria are not sufficiently described	1. No information about the baseline characteristics 2. Allocation procedure is not described 3. Case definition is not sufficiently described 4. Exclusion criteria are not sufficiently described

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Chapter 11

General discussion, future perspectives and conclusions

GENERAL DISCUSSION - LESSONS FROM THE DUTCH EPIDEMIC

Between 2007 and 2010 The Netherlands faced an enormous outbreak of Q fever, a zoonosis caused by the bacterium *Coxiella burnetii*.[1] This outbreak was a catastrophe for both human and veterinary health. Within a period of only four years over 4000 human symptomatic acute Q fever cases were notified, of whom at least 25 died.[2] Furthermore, long-term consequences of acute illness proved to be significant, as shown by a study among 515 acute Q fever patients. Two third of these patients suffered from functional impairment (mainly because of fatigue) and impaired quality of life 12 to 26 months after the onset of illness.[3] Furthermore, hospitals in the epicentrum of the epidemic are increasingly confronted with patients with highly probable and proven chronic Q fever. In the veterinary field culling of thousands of pregnant goats and sheep in the first half of 2010 had an extreme impact on the psychological and financial situation of farmers and their families.[4] Good surveillance and cooperation between the Ministry of Health, Welfare and Sport and the Ministry of Agriculture was crucial to curb the epidemic. Apart from these challenges, the unique Dutch situation gave researchers the opportunity to gain knowledge about different aspects of this relatively rare infectious disease. Since 2008 over twenty PhD programs were started covering many of these aspects. However, research during an epidemic is challenging. On one hand time is scarce because policy makers and clinicians need evidence to support their practices. On the other hand a high level of evidence should be pursued by performing time-consuming prospective trials, so that recommendations can be immediately implemented in practice. Besides this, during the Dutch epidemic, public pressure asked officials to take several immediate actions that were not evidence based at that moment. These actions had a great impact on the epidemic and therefore also on several studies running in 2009 and 2010, the years in which the epidemic was curbed. Finally, besides answers, most of the time research also produces many new questions; a ''flywheel''. This is also what we experienced; we learned a lot about the consequences of Q fever during pregnancy. We got some answers but remained with many new questions too.

The problem – risks of Q fever during pregnancy

Almost all knowledge concerning the effects of Q fever during pregnancy that was available at the start of the Dutch epidemic derived from several case-reports and case series written by the French group of Raoult.[5,6] Their retrospective landmark study, performed between 1991 and 2005, showed a

devastating association between insufficiently treated Q fever during pregnancy – both symptomatic and asymptomatic - and several obstetric and maternal complications.[5] Furthermore in a Canadian cohort study in collaboration with the French group, an association was found between *C. burnetii* seropositivity in parturient women and adverse pregnancy outcomes, further underscoring the risks of asymptomatic infection.[7] Although the results of especially the retrospective study needed to be cautiously interpreted as the study design has probably led to an overestimation of risks, from the start of the Dutch Q fever epidemic pregnant women were considered as a risk group by health authorities and policy makers.[8] When the outbreak still seemed to be located in a very restricted area in 2007, already all pregnant women were informed and offered serological screening.[9] However, based on the available reports at that time, the Health Council of The Netherlands concluded that evidence was insufficient to promote large-scale routine screening when the epidemic spread to adjacent areas the next year. Instead, they ordered that rigorous studies to assess the effectiveness of such serological screening programs amongst pregnant women were required.[8] We subscribed this conclusion in Chapter 3 of this thesis after scrutinising the available evidence with criteria developed by the World Health Organization.

The Dutch outbreak has given several new insights into the *C. burnetii*-associated risks during pregnancy. The first Dutch publication focusing on this subject was published in March 2009. Women in high, middle and low-risk areas for Q fever were serologically screened for the presence of *C. burnetii* antibodies to estimate seroprevalences. Since the majority was screened anonymously, in only three cases with an acute infection data on clinical outcome were available. Two of these three women were treated with cotrimoxazole during the remainder of the pregnancy. All women were asymptomatic, had uncomplicated pregnancies and deliveries. Furthermore, polymerase chain reaction (PCR) for *C. burnetii* DNA on birth products were all negative.[9] Two other case reports of pregnant women with an active infection were published almost two years later. The first concerned an asymptomatic woman who was routinely screened at 38 weeks of gestation because of a Q fever outbreak near her work. Acute *C. burnetii* infection was diagnosed and therefore delivery was induced to prevent possible complications. A healthy infant was born and PCR on birth products was negative.[10] The second case concerned a woman in whom the pregnancy was complicated by a symptomatic chronic Q fever infection, for which she was extensively treated. Finally, also this pregnancy led to the birth of a healthy child (Chapter 2). A

larger study was performed by Van der Hoek et al., who retrospectively screened 1646 sera from pregnant women in Q fever high-risk areas for the presence of immunoglobulin (Ig)M and IgG phase II. Seropositivity, suggesting either previous or recent infection, was not associated with any adverse pregnancy outcome.[11] The results of our clustered randomised controlled trial, presented in Chapter 4, 5 and 6, confirm these results. Absence of significant placental pathology in asymptomatic seropositive cases (Chapter 7) might explain this favourable clinical outcome.

In conclusion, the first lesson we can learn from the Dutch Q fever epidemic is that asymptomatic *C. burnetii* seropositivity during pregnancy turned out not to be as hazardous as was thought based on previous literature. There are three main explanations for the discrepancy between our findings and the available literature. First of all it is hypothesised that differences in virulence between *C. burnetii* strains involved in the different outbreaks exist.[12] Genotyping of Dutch samples is ongoing and shows that at least five strains were involved in the Dutch outbreak, which implies that environmental circumstances (such as high density of farms and people, dry periods in spring) favoured the Dutch Q fever spread rather than that one highly virulent *C. burnetii* strain was responsible.[13] However, since in The Netherlands a relatively high number of chronic Q fever has been described in patients with aneurysms[14], it can be hypothesised that the strains involved are highly virulent for people with underlying vascular diseases at least. On the other hand, it might be hypothesised that pregnant women are relatively protected, although we do not have an explanation for this observation. Secondly, available studies in the literature used several different, mostly non-commercial, serodiagnostic methods and cut-off values, probably influencing sensitivity and specificity (see further). While these first two explanations for the difference in study outcomes are mainly suggestive, a third reason seems most likely. This reason concerns a difference in study design between the landmark study of the group of Raoult[5] and the recent prospective Dutch studies. The retrospective design of the French study may have led to selection bias by testing for *C. burnetii* infection *after* obstetric complications had occurred. This will have led to an overestimation of risks.

The risks of *symptomatic* acute or chronic Q fever during pregnancy remain unclear, particularly because symptomatic pregnant cases are scarce, even after an enormous outbreak like the one in The Netherlands. The assumption that symptomatic Q fever during pregnancy may pose a risk to the pregnant women involved is underscored by the fact that the woman we

describe in Chapter 2 is, to our knowledge, the youngest patient with chronic Q fever described in this epidemic. The published case reports and series focussing on symptomatic cases remain the best available protocols for management of these cases.

The screening population

Since the incidence of Q fever varies largely between regions and within a region varies between different time periods, the number of pregnant women needed to screen, to prevent one complication possibly caused by an infection with *C. burnetii,* fluctuates significantly. Our clustered randomised controlled trial (Chapter 4, 5 and 6) was performed in Q fever high-risk areas based on the incidence of notified Q fever cases in those regions in 2009 and the beginning of 2010. The high percentage of seropositive women (15%) agreed with the high level of endemicity in the study regions. Still, the prevalence of acute infection during the actual study period was very low and probably therefore the screening strategy was ineffective in reducing the risk of obstetric complications in seropositive women within this region. Logically, screening in an even lower-risk population would not be effective at all. Effectiveness in a higher risk population, e.g. pregnant women living within a 2 to 5 kilometre zone around an infected farm[1], with occupational hazard for Q fever or with complicated pregnancies, can not be excluded. Furthermore, we can also not exclude a benefical effect of routine screening of women in their first trimester of pregnancy, since in the clustered RCT (Chapter 4, 5, and 6) screening started at 20 weeks of gestation. However, a recent Danish study showed no association between *C. burnetii* infection and spontaneous abortion up to 22 weeks of gestation[15], indicating that screening earlier in pregnancy would probably also be ineffective.

The test and the treatment

As already mentioned research provides some answers, but also generates many new questions. This proved certainly to be the case in the field of serological assays. The performance (sensitivity and specificity) of serological assays to diagnose acute disease is highly influenced by the incidence of the disease.[16] With respect to the Dutch Q fever epidemic we can differentiate two time periods: the beginning of the outbreak and the end of the outbreak. At the beginning of the epidemic the performance of serological assays proved to be high, because the background seroprevalence was low in combination with a relatively high incidence.[16] At the end of the epidemic, however, the background seroprevalence increased, as shown by de studies in Chapter 5

and 8 of this thesis and by several other studies.[17,18] At the same time the incidence of acute infections decreased. These circumstances in combination with the fact that antibodies against *C. burnetii* remain present for months or even years, impeded interpretation of serological test results, with a possible increase of false-positive results. Besides these facts, the Dutch Q fever outbreak was felt a threat to several groups at risk and led to more awareness for Q fever among health care workers and the public. Therefore, many more (screening)tests were performed by patients with less specific or even without any symptoms. Due to these changes, cut-off values and interpretation of antibody profiles remain subject for debate. Many studies have been published in this field[16,19-23] and its plausible that many more will follow. During pregnancy interpretation of serology is even more complicated, since two opposite theories exist with respect to (the extent of) antibody formation. The first theory includes the hypothesis that during pregnancy sex hormones cause shifts of immunity from cell mediated to humoral, which could lead to higher immunoglobulin levels at baseline and in response to infection.[24,25] The opposite theory assumes that pregnancy causes a relative state of immunosuppression, since an attenuated antibody response against infectious diseases has been described.[26] Possibly, these lower antibodies levels are caused by a higher distribution volume during pregnancy. Overall, it is clear that antibody responses in pregnancy require further investigation.

Randomised studies on the treatment of pregnant women with a *C. burnetii* infection have not been performed yet and probably never will. Since our clustered randomised trial (Chapter 4, 5 and 6) was a pragmatic study, decisions regarding the type and duration of antibiotic treatment in cases with an acute infection were made by the medical specialists involved. Treatment with cotrimoxazole for at least five weeks had been suggested to be the treatment of choice during pregnancy[5] and was given to women in their second trimester of pregnancy (n=3). Women who were diagnosed in their third trimester received erythromycin (n=4), since cotrimoxazole is relatively contraindicated prior to delivery because of neonatal hyperbilirubinemia. Since the number of treated women in our study was small we are unable to draw conclusions about effectiveness and safety of these drugs. Rigorous studies on treatment during pregnancy are needed, mainly focusing on treatment of symptomatic Q fever.

The costs

Since, with the current economic situation, costs of healthcare are of increasing importance, we aimed to assess the cost-effectiveness of routine

screening during pregnancy as well (Chapter 4). However, because there was no relevant clinical beneficial effect of screening, there was refrained of a rigorous cost-effectiveness study. Still, uncertainty in the result of the clinical outcome existed since the 95% confidence interval (CI) of the odds ratio included a risk reduction due to screening up to 40%. Therefore we performed an explorative cost-utility analysis in which we focused on preterm delivery.[27] Input data on the costs of healthcare were based on the Dutch reimbursement system, including the costs of screening, treatment of infected women and costs associated with a hospital delivery.[28,29] Since Dutch data were lacking, costs of preterm delivery at different terms of pregnancy and the risk for severe outcome as well as the corresponding utilities of these outcomes were based on studies from the USA.[30,31] Three outcome conditions were distinguished: normal health (utility=1), severe disability (utility=0.61) and dead (utility=0). The risks for very preterm delivery (<34 weeks of gestation), preterm delivery (<37 weeks of gestation) and at term delivery (≥37 weeks of gestation) per strategy were based on our trial data (1.5%, 3.9% and 94.6% for the intervention group and 1.2%, 4.9% and 93.9% for the control group, respectively). We used a decision-tree model to compare the screening and no-screening (control) strategy, developed and analysed with TreeAge Pro 2011 (TreeAge Software, Williamstown, MA, USA). The intervention strategy turned out to be more costly (+€275.75) and slightly less effective (-0.000043 quality adjusted life years (QALY)); screening was dominated by the no-screening strategy. Probabilistic sensitivity analysis, using Monte Carlo Markov Chain (MCMC) with 10,000 iterations and a willingness to pay (WTP) of €50,000 per QALY, showed a delta net monetary benefit (ΔNMB) of - €274.51 (95% CI - €283.44 to - €265.57). The negative ΔNMB indicates that the screening strategy, under these circumstances and assumptions, is not cost-effective.

No other studies have been reported on the cost-effectiveness of routine screening for *C. burnetii* infection during pregnancy so far. The lessons we can learn from the Dutch epidemic are therefore entirely based on the studies in this thesis. Although, in our cost-utility analysis a part of the input data on costs was based on American data and therefore possibly not entirely applicable to the Dutch situation, we may conclude that the screening strategy was obviously not cost-effective, based on the finding that the screening strategy was dominated by the no-screening strategy.

Overall

Implementation

Since the success of a screening program is not only based on medical effectiveness but also on a successful implementation, willingness of pregnant women to participate is indispensable. In Chapter 5 we observed that despite the fact that the screening study was performed in a Q fever high-risk area the participation rate of pregnant women was low (20%). In Chapter 9 we identified the determinants of pregnant women's decisions regarding participation in a (possible future) Q fever screening and treatment program. Although the intention to participate was already somewhat higher than in the screening trial, still almost 50% of the respondents did not intend to participate in such a program, which is low in comparison with the existing screening program for other infectious diseases during pregnancy.[32] The sole determinant of a higher intended program uptake was a more positive appraisal of program efficacy and convenience. This appraisal in turn was associated with perceived risk and knowledge about Q fever during pregnancy. Since before the recent Dutch epidemic Q fever used to be a rare disease, lack of knowledge about possible consequences of Q fever during pregnancy could have played a major role in refusal of participation in the screening trial (Chapter 4, 5 and 6) or future screening programs (Chapter 9). In conclusion, these findings indicate that, at this moment, the acceptance of a preventive screening program among pregnant women might not be straightforward.

Alternative methods

While discussing the effectiveness of screening it is also important to pay attention to alternative methods to minimise *C. burnetii* associated complications. One of these methods, besides the most important veterinary measures to curb the epidemic[4], could be human vaccination. In Chapter 10 we performed a meta-analysis to assess the effectiveness of human Q fever vaccination. Partly based on this analysis the Health Council of The Netherlands advised the Ministry of Health, Welfare and Sport to start vaccination among patients at risk for chronic Q fever – including patients with cardiac valve diseases, congenital heart diseases, and vascular defects – as part of individual patient care. Vaccination of pregnant women however, was not advised because the producer of the vaccine dissuaded use during pregnancy since safety in this group had never been tested and could not be guaranteed.[33]

In spring 2011 a vaccination campaign among the high-risk cardiac patients was started in the areas with the highest Q fever incidence. General

practitioners and medical specialists identified 2688 patients at risk of whom 907 refused vaccination or turned out to have no proper indication. The remaining 1781 patients received a skin test and were screened for the presence of antibodies against *C. burnetii*, since vaccination of persons who have previously been exposed to *C. burnetii* may lead to serious adverse reactions. Of those patients 394 (22%) had a positive pre-vaccination test and were excluded, again pointing to the massive seroprevalence in this area. Another 21 patients declined. Finally, a total of 1366 patients were vaccinated with the Australian Q-vax® vaccine.[34] Research is ongoing to determine vaccine efficacy and safety.[34,35]

Overall, the human Q fever vaccination campaign is a clear example of immediate implementation of recommendations generated by research performed during the epidemic. Further research is required to determine vaccine efficacy and safety in the specific risk groups.

FUTURE PERSPECTIVES

Q fever and pregnancy

This thesis predominantly focussed on the diagnosis and consequences of asymptomatic *C. burnetii* infection during pregnancy. Still, data on *symptomatic* Q fever during pregnancy remain scarce. Further research, preferably randomised controlled trials, should focus on the risks (both obstetrical and maternal), pathophysiology and treatment of symptomatic Q fever during pregnancy. Since these cases will be scarce, cooperation between different countries facing Q fever outbreaks is indispensible to create a large cohort of affected pregnant women. Central notification of Q fever affected pregnant women could also be useful. Animal models might be helpful in understanding pathophysiological mechanisms. If the risks of symptomatic Q fever during pregnancy turn out to be very high, research about preventive strategies like vaccinating of this specific group should be performed.

Genotyping of *Coxiella burnetii*

To gain insight into the differences in the magnitude and clinical consequences of *C. burnetii* infection between different outbreaks, genotyping of *C. burnetii* strains involved in the Dutch outbreak should be continued. The results should be compared with the genotypes of the strains involved in the previous outbreaks in other countries. When it can be proved that different genotypes have different clinical consequences, this probably will affect future multinational studies in symptomatic women as mentioned earlier.

Long-term consequences

Since the number of notified acute Q fever cases steeply decreased in The Netherlands since 2010, the focus will change from acute illness to long-term consequences of the disease. Reactivation of primary infections during pregnancy has been described in subsequent pregnancies.[5] The incidence, risk factors and pathophysiological mechanisms of these reactivations are unknown. Also data on the follow-up of children from women with Q fever before or during pregnancy is lacking and should be subject of future research. Furthermore, chronic Q fever is estimated to become an enormous health problem, especially in patients with underlying cardiac valve or vascular diseases. Besides that, with the protocolised follow-up of acute Q fever patients, many serological profiles suggesting chronic infection will be found

also in the absence of clinical signs and symptoms. The significance of these antibodies is unclear[36] and requires further investigation. Finally, also other long-term consequences, like chronic fatigue syndrome, need attention. Trials about the effectiveness of different types of treatment of patients with debilitating fatigue are already ongoing.[37]

Prediction models

To optimise individual patient care, prognostic models have to be developed to predict the response to treatment and the risk of complicated outcome after an acute (symptomatic) Q fever infection in both the general population and in risk groups like pregnant women. Prognostic modelling might also be helpful in predicting the magnitude of an outbreak, which is of great importance to policy makers worldwide. Input data for such prediction models are needed but require comprehensive and intensive research because large cohorts of patients with detailed information about predictors are needed.

CONCLUSIONS AND RECOMMENDATIONS

Although the Q fever epidemic was a disaster for both human and veterinary health, the unique Dutch situation gave researchers the opportunity to gain knowledge about different aspects of this relatively rare infectious disease. Asymptomatic *C. burnetii* seropositivity during pregnancy turned out not to be as hazardous as shown by previous research. Routine screening starting at 20 weeks of gestation was not associated with a relevant reduction in obstetric complications in seropositive women and was not cost-effective. Furthermore, the intention of pregnant women to participate in a (future) screening program was low, which indicates that the acceptance of such a preventive program might not be straightforward and supports our notion that screening will probably be an ineffective strategy. Therefore, in the current setting, routine screening for *C. burnetii* infection of pregnant women living in Q fever high-risk areas should not be advised. The risks, treatment and pathophysiology of *symptomatic* acute or chronic Q fever require further investigation. Serodiagnosis of acute and chronic Q fever, especially during pregnancy, is challenging because the performance of serological assays is highly influenced by the a priori chances, cut-off values vary between settings and pregnancy is accompanied by immunological changes. Minimising *C. burnetii* associated complications is possible by human Q fever vaccination. However, in the Dutch vaccination campaign pregnant women were excluded due to lack of knowledge about safety. Although in The Netherlands the number of notified acute Q fever cases steeply decreased since 2010, the Q fever problem still deserves attention in the future, because large-scale long-term consequences including chronic Q fever and chronic fatigue syndrome are expected and other European countries are facing Q fever outbreaks as well. Consequences for women infected with *C. burnetii* during previous pregnancies may become clear during the coming years. Good surveillance and awareness remain of great important to signal possible unexpected consequences – both during pregnancy and in the general population – and new outbreaks.

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Chapter 12

Summary

Q FEVER

Between 2007 and 2010 The Netherlands suffered from on enormous human Q fever outbreak with over 4000 notified cases. Q fever is a zoonosis, caused by the intracellular bacterium *Coxiella burnetii*. In the Dutch situation, especially infections in dairy goat and sheep are hypothesised to be the main sources of human infection. Peron-to-person spread is rare. Acute *C. burnetii* infection is characterised by fever, hepatitis or pneumonia, but remains asymptomatic in 60% of the cases. The incubation period is one to three weeks. Since *C. burnetii* is highly infectious and cut-off values for serodiagnosis are inconsistent, diagnosing Q fever is difficult. Additive in serodiagnosis is the characteristic of *C. burnetii* of antigenetic phase variation. Depending on the duration of infection, antibodies against two phases of antigens are produced (first against phase II antigen, later against phase I). Therefore a distinction can be made between an acute, previous or chronic infection. Treatment of acute Q fever consists of doxycycline for at least two weeks. After an acute infection 1-5% of the patients develop chronic Q fever which is often complicated by endocarditis or infection of vascular structures. The risk of chronic Q fever has been reported to be increased in immunocompromised patients, patients with underlying cardiac valve or vascular diseases and pregnant women. In case of chronic Q fever long-term treatment with doxycycline in combination with hydroxychloroquine is recommended.

PREGNANCY

Besides the possible increased risk of developing chronic Q fever, infection during pregnancy has been associated with obstetric complications including miscarriage, preterm delivery, intrauterine growth restriction, oligohydramnios and foetal death. Long-term treatment with cotrimoxazole (doxycycline and hydroxychloroquine are contraindicated from the second trimester of pregnancy) has been shown to decrease the risk of complicated outcome. Since up to 90% of the pregnant women with a *C. burnetii* infection remain asymptomatic, routine screening during pregnancy in endemic areas for Q fever could be of great value to prevent complications in this high-risk group, but evidence from randomised trials is lacking. The studies presented in this thesis aimed to provide evidence on this topic. Furthermore, related issues were discussed, including specificity of serodiagnosis, placental pathology, risk-perception and human Q fever vaccination.

After a general introduction in **Chapter 1**, this thesis started with a case report concerning a 42-year old pregnant woman with chronic Q fever (**Chapter 2**). The patient suffered from a pneumonia caused by *C. burnetii* shortly before her third pregnancy. During regular serological follow-up 6 months after the primary infection chronic Q fever is diagnosed based on increasing titers of IgG phase I and II and a positive *C. burnetii* PCR in serum. Since there exists a contraindication for treatment with doxycycline, hydroxychloroquine and cotrimoxazole due to pregnancy and an allergy respectively, the patient is treated with erythromycin. She experiences many complaints of dyspnoea, fatigue and weight loss. Therefore at 38 weeks and two days of gestation labor is induced and a healthy boy with a normal birth weight is born. Evidence for endocarditis or infections of vascular structures is not found.

The literature shows that pregnant women have an increased risk to develop chronic Q fever after an acute infection. This is most likely due to the decreased cell-mediated immune response influenced by sex hormones. Furthermore, the placenta seems to be one of the target organs of *C. burnetii*, which might contribute to the increased risk during pregnancy.

In conclusion, because of the increased risk of chronic Q fever, we advise to intensivate serological follow-up of pregnant women with acute Q fever shortly before or during pregnancy to create the possibility for early treatment. *C. burnetii* related obstetric complications do not have to occur, probably as a result of adequate antibiotic treatment.

In **Chapter 3** the Dutch Q fever outbreak and the concerns that raises among pregnant women and their caregivers has been put in a wider perspective. We applied the updated Wilson and Jungner criteria to systematically review the available evidence in the literature for routine screening for *C. burnetii* infection during pregnancy in Q fever high-risk areas. These criteria included, amongst others, judgment of relevance, quality of the screening methods, options for treatment and costs. Because of potential bias in the available studies too much uncertainty remained about the relevance and clinical consequences of *C. burnetii* infection during pregnancy. Furthermore, there was lack of consensus concerning screening methods and treatment options.

Overall, more evidence about the effectiveness of a *C. burnetii* screening program, in addition to other Q fever preventative and controlling measures taken by the European countries, is needed before *C. burnetii* will become a candidate for routine screening during pregnancy.

In **Chapter 4** the study protocol of the clustered randomised controlled trial (RCT) on the (cost)effectiveness of routine Q fever screening during pregnancy has been presented. Primary care midwife centres in Q fever high-risk areas were randomised to recruit pregnant women for either the intervention or for the control group. Randomisation was stratified for the number of goat farms in the municipality and by the size of the midwife centre. Pregnant women, 18 years of age or older, with an estimated date of delivery between June 1st and December 31st 2010 were eligible for inclusion. Women who did not have access to internet and / or an email address, were unable to understand Dutch, unable to give informed consent, or had previously been tested positive for Q fever were excluded. All participating women were asked for a blood sampling between 20 and 32 weeks of gestation. In the intervention group these samples were analysed immediately for antibodies against *C. burnetii* (IgM and IgG, phase I and II) by indirect immunofluorescence assay (IFA). Titres ≥ 1:32 were considered positive. In case of an acute or chronic *C. burnetii* infection, women were referred to an obstetrician, and intensified serological and obstetric follow-up with possible antibiotic treatment according to the local hospital protocol was given. In case of a previous infection serological analysis was repeated in the third trimester of pregnancy to exclude reactivation as part of a chronic infection. Serum samples of the control group were frozen for analysis after delivery similar to the intervention group. In case of a positive test, the participant's general practitioner was advised to perform an extra serological analysis to exclude a chronic infection.

The primary endpoint was a composite measure of maternal (chronic infection) or obstetric complications (a child small for gestational age (SGA), preterm delivery or perinatal mortality) in seropositive women. Secondary outcome measures included fatigue and quality of life one month post partum and costs. In total, we needed 3400 participants to detect a risk reduction of at least 50%, which was defined as being clinically relevant (2-sided, α 0.05, β 0.80). Analyses were performed according to the intention-to-screen principle.

Chapter 5 reported the results of this clustered RCT. Between March and July 2010 55 of the 99 eligible midwife centres were randomised; 27 were allocated to the intervention strategy and 28 to the control strategy. They supervised 6860 eligible pregnant women, of which 1348 (20%) gave informed consent for participation. Of 536 women in the intervention group and 693 in the control group a blood sample was analysed. Fifteen percent in both groups was seropositive (previous or acute infection). Follow-up serology in the intervention group showed that in 77% (23/30) of the cases with a probable acute infection

(IgM present in the first screening sample) antibody titres did not increase and IgM was present as part of a previous infection. In 7 women in the intervention group an acute infection was diagnosed and antibiotic treatment during pregnancy was started. None of the participants in either of the two groups developed a chronic infection.

Screening during pregnancy starting at 20 weeks of gestation did not reduce the risk of obstetric complications in seropositive women (2.2% in the intervention and 1.4% in the control group, odds ratio (OR) 1.54 (95% confidence interval (CI) 0.60-3.96)). Furthermore, there was no difference in the risk of obstetric complications between seropositive and seronegative participants (12% (22/183) and 13% (133/1046) respectively, OR 0.94, 95% CI 0.58-1.52). Participants of the intervention group scored significantly higher on the fatigue score one month post partum compared with the control group (14.6 ± 5.7 versus 13.5 ± 5.5, $P<0.001$).

The incidence of acute Q fever infections steeply declined since 2010. Inclusion of participants in the second half of 2010 would not have been informative and was perceived as unethical. Therefore, we did not reach our projected number of inclusions, but the lower estimate of the 95% CI (OR 0.60) of the primary outcome precludes the a priori defined 50% risk reduction in relevant outcomes.

In conclusion, screening during pregnancy starting at 20 weeks of gestation did not contribute to a relevant reduction of obstetric complications in seropositive women. Therefore, in the current setting, this study does not support routine screening for *C. burnetii* infection of pregnant women living in Q fever high-risk areas.

Chapter 6 went more into depth by focusing on the role of positive *C. burnetii* serology in the prediction of obstetric complications. We aimed to assess the predictive value of *C. burnetii* serological status in addition to well-known risk factors for the development of obstetric complications. Women who participated in the clustered RCT discussed in Chapter 4 and 5 and who were not treated with antibiotics for a *C. burnetii* infection were included. An obstetric complication was defined as any SGA, preterm delivery and/or perinatal mortality. We used multiple logistic regression analysis to build two prediction models; in the base model we only included well-known risk factors like smoking, maternal age and obstetric history; secondly, we added *C. burnetii* serological status, to determine the contribution of this variable. The performance of the prediction models was assessed using receiver operating

characteristic (ROC) analysis and calibration. The validity of the two models was evaluated by bootstrap analysis.

Overall, 1221 women were included, of which 152 (12.4%) developed an obstetric complication. The base model including well-known risk factors had good calibration (Hosmer-Lemeshow test *P*=0.49), but low predictive capacity (area under the ROC-curve (AUC) 0.68; 95% CI 0.63-0.72). Bootstrapping showed an AUC of 0.65, indicating good validity and absence of strong overfitting. Addition of *C. burnetii* serological status to the model did not improve its predictive value.

In conclusion, prediction of adverse obstetric outcomes in a first line, low-risk, healthy population is difficult. Knowledge of *C. burnetii* serological status does not contribute to a better prediction.

The aim of **Chapter 7** was to describe placental histopathology and clinical outcome in women with asymptomatic *C. burnetii* infection during pregnancy and to compare these cases with cases described in the literature. Five women with asymptomatic *C. burnetii* infection during pregnancy could be selected from the clustered RCT (Chapter 4 and 5). Placental examination showed a few scattered fibrotic villi, which could be a result of interruption of foetal blood flow or destruction of capillaries due to previous villitis. In none of the placentas *C. burnetii* could be detected with PCR. Four out of five women delivered at term from living children. The literature search resulted in four cases; all symptomatic. Severe placenta pathology including necrosis and active inflammation was described. Furthermore, in all four placenta's *C. burnetii* could be detected. All pregnancies ended preterm and in two cases perinatal mortality occurred.

In conclusion, asymptomatic and symptomatic *C. burnetii* infection during pregnancy are different entities with respect to placental histopathology and the risk of obstetric complications.

Since acute Q fever during pregnancy is an indication for long-term antibiotic treatment, accurate IgM phase II assays are indispensable. The objectives of the study presented in **Chapter 8** were therefore to describe the seroprevalences of the different antibodies (IgM and IgG, phase I and II) during pregnancy determined with IFA in a low and high-risk area for Q fever, and to estimate the specificity of the IgM phase II IFA during pregnancy. Samples from women from high-risk areas (n=1229) were selected from the clustered RCT (Chapter 4 and 5). Samples from women from low-risk areas (n=180) were obtained anonymously from the Centre for Infectious Diseases

Friesland Izore, The Netherlands, which stores sera of pregnant women drawn routinely as part of a screening program for infectious diseases. IFA was performed in one laboratory using a cut-off titre of 1:32.

The overall *C. burnetii* seroprevalence in both groups of pregnant women from a high-risk and a low-risk area was high, being 15.2% and 11.1% respectively, mainly caused by a high prevalence of IgG phase II. All cases from low-risk areas were IgM phase II negative in contrast to a 4.3% prevalence in the high-risk areas (P=0.001), which indicates 100% specificity of IFA in the detection of IgM phase II, using a cut-off titre of 1:32.

The aim of **Chapter 9** was to identify the determinants of pregnant women's decisions regarding participation in a possible future Q fever screening and treatment program. Therefore, 148 pregnant women living in Q fever high-risk areas filled out a questionnaire containing items concerning health behavior. Questions included, amongst others, intention to participate, Q fever exposure risk, perceived Q fever risk, trust in health professionals and authorities and disease-related knowledge. Fifty-six percent of the respondents intended to participate in the future screening and treatment program. The sole determinant of a higher intended program uptake was a more positive appraisal of program efficacy and convenience. This appraisal was in turn associated with perceived risk and knowledge.

In conclusion, women's appraisal of program efficacy and convenience, their disease-related knowledge and perceived Q fever risk seem to be crucial for their intended program uptake. A successful implementation of a possible future Q fever screening and treatment program may thus depend on these determinants.

In **Chapter 10** we performed a meta-analysis to determine the effectiveness of human Q fever vaccination. Seven studies could be included, describing in total 3752 vaccinees and 1649 nonvaccinees, of which 9 and 81, respectively, developed Q fever. We calculated separate relative risks, the pooled Mantel-Haenszel risk ratio (mhRR) and vaccines effectiveness ((1 − mhRR) × 100%). Furthermore, we assessed the amount of bias. Although the separate and the pooled estimates showed a very high vaccine effectiveness (91-100% and 97%, respectively), conclusions for the general population or of specific groups at risk, like pregnant women, cannot be confidently drawn due to the selected group of participants (mainly abattoir workers) and the potential flaws in the design and report of the studies.

Chapter 13

Nederlandse samenvatting

Q-KOORTS

Van 2007 tot 2010 kampte Nederland met een Q-koortsuitbraak van ongekende omvang, met meer dan 4000 humane gevallen. Q-koorts is een infectieziekte die wordt veroorzaakt door de bacterie *Coxiella burnetii*. Het is een zoönose, wat betekent dat de bacterie verspreid wordt van dieren op mensen. Met name melkgeiten en –schapen zijn de bron van de humane besmettingen in Nederland. Besmetting van mens-op-mens is zeer zeldzaam. Een acute *C. burnetii* infectie wordt gekenmerkt door koorts, hepatitis of pneumonie, maar verloopt in 60% van de gevallen asymptomatisch. De incubatietijd is één tot drie weken. Het stellen van de diagnose Q-koorts is lastig, temeer omdat *C. burnetii* hooginfectieus is en afkapwaarden voor serodiagnostiek niet vaststaan. Bijdragend in de diagnostiek is de antigene variatie die *C. burnetii* vertoont. Afhankelijk van de duur van infectie produceert het lichaam antistoffen tegen antigenen in een bepaalde fase (eerst tegen fase II antigenen, vervolgens tegen fase I). Hierdoor is er een onderscheid te maken tussen een doorgemaakte infectie, acute infectie of chronische infectie. Na het stellen van de diagnose acute Q-koorts, bestaat de eerste keuze behandeling uit een kuur doxycycline voor minimaal twee weken. Een acute infectie leidt in 1-5% van de gevallen tot een chronisch ziektebeeld, waarbij endocarditis of infecties van vasculaire structuren kunnen ontstaan. De kans op het ontwikkelen van chronische Q-koorts wordt groter geacht bij immuungecompromitteerden, patiënten met pre-existent klep- of vaatlijden en zwangeren. Een langdurige behandeling met doxycycline gecombineerd met hydroxychloroquine is in het geval van chronische Q-koorts aangewezen.

ZWANGERSCHAP

Naast het waarschijnlijk verhoogde risico op chronische Q-koorts, veroorzaakt een *C. burnetii* infectie bij zwangeren mogelijk ook risico's voor de foetus, voornamelijk bij infecties vroeg in de zwangerschap. Er zijn aanwijzingen in de internationale literatuur dat onbehandelde besmette zwangeren een verhoogd risico hebben op een miskraam, vroeggeboorte, groeivertraging, oligohydramnion en intra-uteriene vruchtdood. Een langdurige behandeling met co-trimoxazol (doxycycline en hydroxychloroquine zijn gecontraïndiceerd vanaf het tweede trimester van de zwangerschap) zou mogelijk dit risico kunnen verlagen.

Aangezien een *C. burnetii* infectie bij zwangeren in tot wel 90% van de gevallen asymptomatisch verloopt, zou routinematig screenen van zwangeren in risicogebieden voor Q-koorts mogelijk bij kunnen dragen aan een afname van Q-koorts gerelateerde complicaties. Gerandomiseerd onderzoek naar de effectiviteit van een dergelijke screening ontbreekt echter. Met de studies in dit proefschrift hebben we getracht dit vraagstuk op te lossen. Daarnaast kwamen er gelieerde facetten aanbod, waaronder de betrouwbaarheid van diagnostiek, placentapathologie, risicoperceptie en humane Q-koorts vaccinatie.

Na een algemene introductie in **Hoofdstuk 1**, beschreven we in **Hoofdstuk 2** een casus betreffende een 42-jarige zwangere vrouw met chronische Q-koorts. Deze patiënte maakte vlak voor haar derde zwangerschap een pneumonie door veroorzaakt door *C. burnetii*. Bij de reguliere serologische follow-up 6 maanden na de primaire infectie werd de diagnose chronische Q-koorts gesteld op basis van sterk stijgende IgG titers en een positieve *C. burnetii* PCR in serum. In verband met een contra-indicatie voor doxycycline, hydroxychloroquine en co-trimoxazol wegens de zwangerschap en een allergie, respectievelijk, werd de patiënte behandeld met erytromycine. Patiënte ervoer veel klachten van dyspnoe, vermoeidheid en gewichtsverlies. Op maternale indicatie werd de bevalling daarom bij een amenorroeduur van 38 weken en 2 dagen ingeleid. Patiënte beviel uiteindelijk middels sectio caesarea van een gezonde zoon van 3850 gram. Aanwijzingen voor endocarditis of vasculaire infecties werden niet gevonden.

Literatuuronderzoek laat zien dat het risico op een chronische Q-koorts infectie tijdens de zwangerschap waarschijnlijk verhoogd is in verband met een afname van de celgemedieerde immuunrespons onder invloed van oestrogenen en progestagenen. Daarnaast is de placenta één van de doelwitorganen van *C. burnetii*, wat mogelijk bijdraagt aan het verhoogde risico bij zwangeren.

Concluderend adviseren wij in verband met een verhoogd risico op chronische Q-koorts tijdens de zwangerschap, ook na een acute infectie vlak vóór de zwangerschap, de serologische controles te intensiveren, zodat indien noodzakelijk tijdig gestart kan worden met antibiotica. *C. burnetii* gerelateerde zwangerschapscomplicaties, zoals vroeggeboorte en groeivertraging, hoeven niet op te treden, mogelijk ten gevolge van adequate therapie.

In **Hoofdstuk 3** werd het onderwerp Q-koorts tijdens de zwangerschap in een breder perspectief geplaatst. We probeerden, onderbouwd met literatuur, de

vraag te beantwoorden of het zinvol is om zwangeren woonachtig in risicogebieden voor Q-koorts routinematig serologisch te screenen op een infectie met *C. burnetii*. Dit deden we aan de hand van de Wilson en Jungner criteria. Deze criteria werden in 1968 opgesteld om screeningsmethoden systematisch te kunnen beoordelen op onder andere relevantie, kwaliteit van opsporingsmethode, behandelbaarheid en kosten-baten. In de afgelopen jaren verschenen aanvullingen op deze criteria waarbij onder andere de geïnformeerde eigen keuze tot deelname en programma-evaluatie toegevoegd werden. Ook deze aanvullende criteria namen we mee in onze beoordeling.

Vanwege potentiële bias in de beschikbare studies bestond er teveel onzekerheid over de incidentie en de gevolgen van onbehandelde *C. burnetii* infectie tijdens de zwangerschap. Daarnaast was er geen consensus over (de interpretatie van) de optimale screeningsmethode en behandeling.

Concluderend is er meer onderzoek nodig naar de effectiviteit van een *C. burnetii* screeningsprogramma voordat deze infectieziekte een onderdeel zou kunnen worden van het huidige infectieziektescreenings-programma tijdens de zwangerschap.

In **Hoofdstuk 4** beschreven we het studieprotocol van de geclusterde gerandomiseerde gecontroleerde studie (RCT) naar de (kosten)effectiviteit van screenen op *C. burnetii* infectie tijdens de zwangerschap. Eerstelijns verloskundigenpraktijken in hoogrisicogebieden voor Q-koorts werden gerandomiseerd toegewezen aan de interventie of controle strategie. De randomisatie was gestratificeerd voor het aantal geitenbedrijven in de omgeving en de grootte van de verloskundigenpraktijk. Zwangere vrouwen onder controle van de deelnemende praktijken, van 18 jaar of ouder en met een a terme datum tussen 1 juni 2010 en 31 december 2010 waren geschikt voor inclusie. Vrouwen zonder toegang tot internet / e-mail, die eerder Q-koorts positief waren getest, geen begrip hadden van de Nederlandse taal of geen geïnformeerde toestemming konden geven, werden geëxcludeerd.

Alle deelnemende zwangeren werd verzocht om een buisje bloed af te laten nemen bij een amenorroeduur tussen 20 en 32 weken. Bij zwangeren onder controle bij een interventiepraktijk werd het serum direct geanalyseerd op te aanwezigheid van *C. burnetii* antistoffen (IgM fase I en II en IgG fase I en II) door middel van indirecte immunofluorescentie assay (IFA). Titers ≥ 1:32 werden beschouwd als positief. In het geval van een acute of chronische infectie werd de zwangere vrouw verwezen naar een obstetricus voor serologische follow-up met eventuele antibiotische therapie volgens

ziekenhuisprotocol en geïntensiveerde zwangerschapscontroles. In het geval van een doorgemaakte infectie verrichtte de verloskundige een extra serologische controle in het derde trimester van de zwangerschap ter uitsluiting van een chronische infectie. De sera van zwangeren die onder controle waren bij een controlepraktijk werden ingevroren en post partum geanalyseerd, conform de methode van de interventiegroep. In geval van een positieve test werd de huisarts geadviseerd om een extra serologische controle te verrichten ter uitsluiting van een chronische infectie.

De primaire uitkomstmaat was een samengestelde uitkomstmaat van een maternale (chronische infectie) of obstetrische complicatie (vroeggeboorte, dysmaturiteit of perinatale sterfte) bij *C. burnetii* seropositieve zwangeren. Secundaire uitkomstmaten betroffen moeheid en kwaliteit van leven één maand post partum en kosten. In totaal waren er 3400 zwangeren nodig om een reductie van de primaire uitkomstmaat van 50% aan te tonen, het op voorhand vastgestelde klinisch relevantie verschil (2-zijdige test, α 0,05, β 0,80). Analyses werden uitgevoerd volgens het intention-to-screen principe.

In **Hoofdstuk 5** werden vervolgens de resultaten besproken van deze geclusterde RCT. Tussen maart en juli 2010 werden er 55 van de 99 verloskundigenpraktijken in risicogebieden voor Q-koorts gerandomiseerd toegewezen; 27 lootten voor de interventiestrategie en 28 voor de controlestrategie. Alle praktijken tezamen superviseerden 6860 zwangeren die voldeden aan de inclusiecriteria, van wie er 1348 (20%) wilden deelnemen. Vijfhonderdzesendertig zwangeren in de interventiegroep en 693 in de controlegroep lieten een bloedmonster afnemen, waarvan er in beide groepen 15% *C. burnetii* seropositief waren (doorgemaakte of acute infectie). Serologische follow-up in de interventiegroep toonde aan dat bij 77% (23/30) van de zwangeren met een verdenking op een acute infectie (IgM aanwezig in het eerste screeningsmonster) antistoftiters niet doorstegen en IgM aanwezig was in het kader van een doorgemaakte infectie. Bij 7 zwangeren in de interventiegroep (1,3%) werd de diagnose acute *C. burnetii* infectie gesteld en volgde een behandeling met antibiotica tijdens de zwangerschap. Geen van de zwangeren in beide groepen ontwikkelde een chronische infectie.

Screenen tijdens de zwangerschap startend bij een amenorroeduur van 20 weken verlaagde het risico op obstetrische complicaties bij seropositieve zwangeren niet (2,2% in de interventiegroep en 1,4% in de controlegroep, odds ratio (OR) 1,54, 95% betrouwbaarheidinterval (BI) 0,60-3,96). Daarnaast verschilde het percentage obstetrische complicaties tussen seropositieve en seronegatieve zwangeren niet (12% (22/183) en 13%

(133/1046) respectievelijk, OR 0,94, 95% BI 0,58-1,52). Zwangeren in de interventiegroep waren significant vermoeider één maand post partum dan de zwangeren in de controlegroep (score 14,6 ± 5,7 versus 13,5 ± 5,5, *P*<0,001).

In 2010 daalde de incidentie van acute Q-koorts aanzienlijk. Inclusie van zwangeren in de tweede helft van 2010 zou daarom niet informatief zijn en werd als onethisch verondersteld. Daarom werd het beraamde aantal inclusies niet gehaald. Echter de laagste schatting van het 95% BI (OR 0,60) van de primaire uitkomstmaat sluit de op voorhand gedefinieerde klinisch relevante complicatiereductie van 50% uit.

Concluderend leidt screenen tijdens de zwangerschap, startend bij een amenorroeduur van 20 weken, niet tot een relevante complicatiereductie bij seropositieve zwangeren. In de huidige situatie adviseren wij daarom om niet routinematig te screenen op de aanwezigheid van *C. burnetii* antistoffen bij zwangeren woonachtig in hoog risicogebieden voor Q-koorts.

In **Hoofdstuk 6** werd de betekenis van positieve *C. burnetii* serologie tijdens de zwangerschap verder uitgediept. Het doel van de studie was om te bepalen wat de additief voorspellende waarde was van de aanwezigheid van *C. burnetii* antistoffen tijdens de zwangerschap, naast bekende risicofactoren, op het ontwikkelen van obstetrische complicaties. Zwangeren die deelnamen aan de geclusterde RCT (Hoofdstuk 4 en 5) die niet behandeld waren met antibiotica voor een *C. burnetii* infectie, werden geïncludeerd. Een obstetrische complicatie was gedefinieerd als het optreden van vroeggeboorte en / of dysmaturiteit en / of perinatale sterfte. Er werden twee modellen gemaakt met behulp van meervoudige logistische regressie; in het eerste model werden alleen bekende risicofactoren zoals roken, maternale leeftijd en obstetrische voorgeschiedenis meegenomen; in het tweede model voegden we *C. burnetii* serologische status toe om de bijdrage van deze variabele te bepalen. De prestatie van de prognostische modellen werd beoordeeld door middel van receiver-operating-characteristic (ROC) analyse en een kalibratie. Interne validatie werd verricht door middel van bootstrapping.

In totaal werden er 1221 vrouwen geïncludeerd, van wie er 152 (12,4%) een obstetrische complicatie kregen. Het model met de bekende risicofactoren had een goede kalibratie (Hosmer-Lemeshow test *P*=0,49), maar de voorspellende capaciteit was laag (oppervlakte onder de ROC-curve (AUC) 0,68, 95% BI 0,63-0,72). Bootstrapping toonde met een AUC van 0,65, dat de interne validatie goed was en er van een sterke overfitting geen sprake

was. Het toevoegen van *C. burnetii* serologische status verbeterde de voorspellende capaciteit niet.

Concluderend is het voorspellen van obstetrische complicaties in een cohort relatief gezonde eerstelijns zwangeren in hoogrisicogebieden voor Q-koorts lastig. Kennis over *C. burnetii* antistofstatus draagt niet bij aan een betere voorspelling.

Het doel van de studie beschreven in **Hoofdstuk 7** was om de placenta histopathologie en de klinische uitkomsten te beschrijven van zwangeren die een asymptomatische *C. burnetii* infectie tijdens de zwangerschap hadden doorgemaakt en om deze resultaten te vergelijken met casus uit de literatuur. Vijf zwangeren met een asymptomatische *C. burnetii* infectie tijdens de zwangerschap konden worden geselecteerd uit de geclusterde RCT (Hoofdstuk 4 en 5). Histopathologisch onderzoek van de placenta's toonde enkele fibrotische villi, wat een gevolg kan zijn van interruptie van de foetale doorbloeding of van destructie van capillairen ten gevolge van een doorgemaakte villitis. In geen van de placenta's kon *C. burnetii* worden aangetoond middels PCR. Vier van de vijf zwangeren bevielen a term van een levend kind. Literatuuronderzoek resulteerde in vier casus; allen symptomatisch. Ernstige placentapathologie waaronder necrose en actieve inflammatie werd beschreven. Tevens kon in alle vier de placenta's de aanwezigheid van *C. burnetii* worden aangetoond. Alle zwangerschappen eindigden preterm, waarbij er in twee gevallen sprake was van perinatale sterfte.

Concluderend zijn asymptomatische en symptomatische *C. burnetii* infectie tijdens de zwangerschap twee verschillende entiteiten ten aanzien van placentapathologie en het risico op obstetrische complicaties.

Aangezien acute Q-koorts tijdens de zwangerschap een indicatie is voor langdurige antibiotische therapie, zijn betrouwbare diagnostische methoden, met name voor het aantonen van IgM fase II, onmisbaar. De doelen van de studie gepresenteerd in **Hoofdstuk 8** waren daarom om de seroprevalenties van de verschillende antistoffen (IgM fase I en II en IgG fase I en II) bij zwangeren woonachtig in een hoog- en laagrisicogebied voor Q-koorts te bepalen met IFA en om een indruk te krijgen over de specificiteit van de IgM fase II IFA. Serum van zwangeren uit hoogrisicogebieden (n=1229) werd verkregen vanuit de geclusterde RCT (Hoofdstuk 4 en 5). Serum van zwangeren woonachtig in laagrisicogebieden (n=180) werd anoniem verkregen van Izore Centrum Infectieziekten Friesland. Dit centrum bewaart serum van zwangeren dat routinematig is verkregen als onderdeel van het

landelijke screenings-programma naar infectieziekten tijdens de zwangerschap. IFA werd verricht in één laboratorium gebruikmakende van een cut-off titer van 1:32.

De totale *C. burnetii* seroprevalentie in zowel de zwangeren uit hoogrisicogebieden als in de zwangeren uit laagrisicogebieden was hoog - 15,2% en 11,1%, respectievelijk – en werd voornamelijk veroorzaakt door een hoge prevalentie van IgG fase II. Alle zwangeren uit het laagrisicogebied waren IgM fase II negatief, in tegenstelling tot een 4,3% IgM fase II prevalentie in de hoogrisicogebieden (*P*=0,001). Hieruit blijkt dat IFA 100% specifiek is in de detectie van IgM fase II, indien er gebruik wordt gemaakt van een cut-off titer van 1:32.

Het doel van **Hoofdstuk 9** was om determinanten te identificeren die bepalend zijn in de beslissing van zwangere vrouwen om wel of niet deel te nemen aan een mogelijk toekomstig screen-en-behandel programma voor Q-koorts. Hiertoe vulden 148 zwangere vrouwen woonachtig in hoogrisicogebieden voor Q-koorts een vragenlijst in met items, geselecteerd op basis van de literatuur, aangaande gezondheidsgedrag. De vragen betroffen onder andere intentie tot participatie, het blootstellingsrisico, risicoperceptie, vertrouwen in zorgprofessionals en de overheid en ziekte-gerelateerde kennis. Zesenvijftig procent van de respondenten gaf aan deel te willen nemen aan het mogelijk toekomstige screen-en-behandel programma. De sleuteldeterminant voor intentie tot deelname was een positieve beoordeling van het programma aangaande werkzaamheid en gemak. Deze positieve beoordeling was geassocieerd met risicoperceptie en kennis.

Concluderend zijn een positieve beoordeling van programma-werkzaamheid en –gemak, ziektegerelateerde kennis en Q-koorts specifieke risicoperceptie van zwangeren cruciaal voor de intentie tot deelname aan een screen-en-behandel programma. Om een succesvolle implementatie van een dergelijk programma te bewerkstelligen, dient daarom rekening te worden gehouden met deze determinanten.

In **Hoofdstuk 10** werd door middel van een meta-analyse de effectiviteit van humane Q-koorts vaccinatie onderzocht. Zeven studies konden worden geselecteerd, waarin in totaal 3752 mensen werden gevaccineerd en 1649 mensen ongevaccineerd waren. Acht gevaccineerden ontwikkelde Q-koorts ten opzichte van 91 ongevaccineerden. We berekende afzonderlijke relatieve risico's, de gepoolde 'Mantel-Haenszel risk ratio (mhRR)' en vaccineffectiviteit ((1−mhRR) × 100%). Tevens werd de mate van bias beoordeeld. Ondanks dat

de afzonderlijke en gepoolde vaccineffectiviteit zeer hoog waren (91-100% en 97%, respectievelijk), kunnen er geen conclusies over de vaccineffectiviteit in de algemene bevolking of in specifieke risicogroepen, zoals zwangere vrouwen, getrokken worden. Dit in verband met de zeer selecte onderzoekspopulatie (met name slachthuismedewerkers) en de gebreken in de onderzoeksopzet en –rapportage.

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Janna Munster was born in Goes, The Netherlands, on March 22, 1985. She grew op with her parents, two brothers and one sister in Terwolde and Lettele, two small villages near the city of Deventer. She received her pre-university secondary education degree at the Etty Hillesum Lyceum Deventer in 2003. She started Medical School at the University of Groningen in the same year. As a student Janna performed a research project at the department of Nephrology and Lungtransplantation of the University Medical Center Groningen under supervision of Prof. dr. Navis, Prof. dr. Van Son, Dr. Seelen and Dr. Van der Bij. During her clinical rotations at the University Medical Center Groningen and the Medical Spectrum Twente, which took place between 2007 and 2009, Janna had a special interest in Obstetrics and Gynaecology. She performed two additional rotations in Obstetrics and Gynaecology in Paramaribo, Suriname, and in Paediatric Surgery in Capetown, South-Africa. She received her medical degree, ''cum laude'', in December 2009. Then, she started this PhD project on Q fever during pregnancy at the University Medical Center Groningen in close collaboration with the Jeroen Bosch Hospital. She was supervised by Prof. dr. Hak, Prof. dr. Aarnoudse and Dr. Leenders. In 2012 Janna started her residency in Obstetrics and Gynaecology at the Medical Spectrum Twente. She will continue her training to become a gynaecologist in the next coming years at the University Medical Center Groningen and the Martini Hospital Groningen.

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